# 17<sup>th</sup> European Weed Research Society Symposium EWRS 2015 22-26 June 2015 – Montpellier SupAgro - France

The 17th European Weed Research Society Symposium was organized with:



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It is the first time since the EWRS was created, in a joint meeting of the EWRC and COLUMA at the UNESCO in Paris in December 1975, that EWRS organizes its main Society Symposium in France. Forty years later, both EWRS and the COLUMA group of AFPP are happy to welcome you at the 17<sup>th</sup> EWRS Symposium in Montpellier, France.



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# **INVITED KEYNOTES**

#### **BIG DATA ANALYTICS FOR WEED CONTROL AND CROP PROTECTION**

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Weed control and crop protection in precision agriculture rely on optical sensors, such as multispectral, hyperspectral or fluorescence cameras. These sensors can be mounted on tractors, robotic ground vehicles or drones, manned aircrafts or on satellites. The data collected with these sensors is the basis for spatially variable application of pesticides, in real time or using map-based information. They are to be combined with other data on local or site specific soil and climate conditions in control strategies and methods.

The large amount of data on weeds, pests and diseases (time, place, density, kind of weed/pest/disease) that is gathered in this way has many potential uses, besides the immediate use of control. In the case of weeds, where many species occur only locally or spread from a center of infestation, targeted weed control becomes possible when a farmer knows where new foci occur and whether that relates to the local soil conditions; presence of trails and waterways; cropping history; and whether current foci are spreading. A farmer can learn whether his/her method of weed control is effective by studying weed infestation levels over the course of years, and by comparing with neighboring fields. From time to time, new weeds become manifest, or weeds that were there already become more prominent, for example as a consequence of the changing climate. This process can be monitored by analyzing data on a regional, national or international scale.

These potential uses of spatial weed, pest and disease data are currently not realized because data are mostly not stored and because farmers and their advisors lack the tools to handle large amounts of data. It is not a lack of available data that impedes progress, but rather the challenge to make proper use of the available data. We argue that a new conceptual model for weed control should be developed which consisting of three elements: (i) store data, (ii) analyze data, and (iii) present information.

A typical sensor generates an electronic signal which is excellently suitable for automatic storage. It should become standard practice that sensors in agriculture are connected to the internet (IoT) and transmit their measurements to permanent storage. This connection can be intermittent, meaning that data are stored locally (on the tractor) until the sensor is within reach of a wireless (WiFi, 4G) network. Establishing the connection and transmitting the data should occur without intervention by the farmer. The transmitted data should be described fully (in terms of what, how, when, where, by whom, what units) and unambiguously. Semantic technologies allow data to be described in such a way that they can be processed both by humans and by machines. Several current Farm Management Information Systems that can serve as permanent storage facilities are already available and provide real time data collection, automatic generation of spatial maps, alerting according to climate critical conditions and adaptive decision making.

Finally, research must develop towards the interaction between real time data collection on weed occurrence, disease and pests and climatic conditions during the growing seasons. These data should be the basis for building models for the physiology and behavior of crop pests at given climatic conditions.

#### A HORIZON SCAN FOR RESEARCH PRIORITIES IN WEED ECOLOGY, EVOLUTION AND MANAGEMENT

<u>Neve P.<sup>1</sup></u>, Barney J.N., Buckley Y., Cousens R.D., Graham S., Jordan N.R., Lawton-Rauh A., Liebman M., Mesgaran M.B., Schut M., Shaw J., Storkey J., Baraibar B., Baucom R.S., Chalak M., Childs D.Z., Christensen S., Eizenberg H., Fernández-Quintanilla C., French F., Harsch M., Heijting S., Harrison L., Loddo D., Macel M., Maczey N., Merotto Jr. A., Mortensen D., Necajeva J., Peltzer D.A., Recasens J., Renton M., Riemens M., Sønderskov M., Williams M.

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In June 2014, the EWRS contributed to sponsorship of a weed ecology workshop held in Benasque, Spain. The workshop was attended by 35 international scientists working in diverse disciplines including weed ecology, weed management, invasion ecology, evolutionary biology and social sciences. The four-day workshop provided participants with the opportunity to reflect on current challenges and approaches in weed ecology, evolution and management.

As part of the workshop format, participants were asked to submit three to five research questions which they considered were major challenges and research needs for the discipline over the next five to ten years. A total of 127 unique questions were submitted. During the workshop, these questions were reviewed, discussed and ultimately distilled into a series of generic and specific research challenges that might help to ensure sustained progress towards sustainable and resilient weed management systems, underpinned by a greater understanding of the ecology and evolution of weeds. The results of this horizon scanning exercise will be presented.

A consensus emerged that future weed management approaches should emphasise systemsthinking and design. Technological innovation will continue to be critical for success, though with a requirement for an ever greater integration of ecological and evolutionary principles in weed management. Research questions and management approaches must take account of important issues of scale (spatial, temporal and institutional), as well as interactions between weeds and other biological taxa. Understanding the potential for weeds to rapidly adapt to management systems, novel environments and global change will be an important element contributing to longterm success in weed management. Importantly, research must be transdisciplinary, involving ecologists, evolutionary biologists, plant scientists, conservationists, agronomists, farmers and social scientists; and must proactively seek stakeholder engagement in tackling and solving global weed management issues.

The converging pressures of global food security and global change, increasing rates of biological invasion, changing regulation, the evolution of herbicide resistance, biodiversity loss and environmental protection present a number of urgent and exciting challenges. Workshop participants noted that many conceptual advances in plant population ecology, invasion ecology and ecological genetics have been motivated by the study of weeds. These disciplines together with advances in 'omics technologies, quantitative biology and weed control technology should be harnessed to ensure a progressive, thriving discipline of 'weed science' motivated to address issues of critical societal importance.

# Translating research into an Integrated Weed Management Program: sharing our company experience

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The challenge of feeding a growing global population – over 9 billion by 2050 – is being made more difficult by three long-term trends: urbanization and the associated loss of arable land; reduced yields due to climate change; and major agronomic challenges such as the spread of herbicide-resistant weeds with their negative impact on agricultural productivity. When surveying farmers about their agricultural practices on the main broad acre rotations, it appears that integrated weed management principles are still relatively unknown or neglected and not broadly implemented. The adoption of these weed best management practices at the farm level requires energy, time, knowledge and long-term investments. The tools that form the basis of weed resistance management programs are based upon diversity in order to disrupt the life cycle of key target weeds. In order to engage farmers, Bayer CropScience set up a global communication campaign "Diversity is the Future" and local initiatives like "Respect the Rotation" in the US, the "Black-Grass Task Force" in the UK or "Diversity Can't Wait" in Australia to increase weed resistance awareness and promote diversity in herbicides, in modes of action, in crops, in herbicide tolerance traits, as well as in complementary non-chemical weed management practices.

Bayer CropScience has set up local activities to educate and inform key stakeholders and demonstrate Integrated Weed Management in practice. The scientists and specialists employed at the Bayer Weed Resistance Competence Center (WRCC) in Frankfurt, Germany, test and develop new solutions to manage resistance and share this knowledge with the international agricultural community and endeavor to broaden partnerships with leading universities and institutes. By better understanding mechanisms of weed resistance development, the solutions are more robust. They search constantly for innovations not only for new crop protection products but also explore the potential for developing new, locally adapted services and solutions. Over the last ten years, the center has worked on resistance projects in 44 countries across Europe, North and South America, Australia, South Africa and Asia Pacific. Looking ahead, the WRCC plans to further broaden cooperation and dialog with external partners.

Bayer CropScience is a member company of Herbicide Resistance Action Committee (HRAC), which is supported by Crop Life International. The HRAC is an international body founded by the agrochemical industry to supporting a cooperative approach to the management of herbicide resistance. Within HRAC, its members are working together to foster a responsible attitude to herbicide use, to communicate herbicide resistance management strategies and support their implementation through practical guidelines.

#### ECOLOGY THEORY MEETS AGRONOMY

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I present the benefits of studying weed communities, and cropping systems more broadly, through an explicitly ecological lens. Trait-based ecology offers an operational framework to compare organisms, communities and ecosystems by treating the biota as a continuous distribution of functional traits. This approach provides a better understanding, and potentially a higher ability to predict, the structure and dynamics of weed communities. In turn, weeds provide an excellent study system for basic ecological research to allow novel tests of Ecological Strategies. Throughout, I highlight how ecological theory or techniques can yield new insights into the management of weed communities with a focus on: individual plant traits, integrated phenotypes, and species interactions.

Our understanding of weed communities comes largely from population biology. However, the quantification of community-wide species interactions through the quantification of all possible pairwise combinations is practically infeasible because of the 'malediction' of dimensionality. The difficulty of this task resides also in the fact that so many different types of (positive and negative) species interactions can simultaneously occur within communities. Several alternative attempts have been proposed in Ecology to frame a pragmatic and operational approach to the quantification of community-wide species interactions. The trait-based characterization of competitive ability of species - i.e. the search for organismal traits able to explain how species' performance influences (or is influenced by) the performance of competing neighbours – paved the road of a generic description of species interactions. In the last decade, this approach has been widely applied to approach community-wide species interactions through the systematic evaluation of the community-level distribution of interaction traits. I discuss the benefits of this approach in a weed management perspective.

In a community ecology perspective, functional traits are expected to reveal assembly processes, under the hypothesis that trait and niche axes co-vary. As such, a predictive framework to trait-based community ecology can be envisioned, with direct applied perspectives for several fields - notably agronomy. However species coexistence and local abundances, two regularly-mentioned targets of predictive community ecology may be elusive, at least at local scale. Overall, I will present the promises and limits of predictive trait-based community ecology using the precision-realism-generality triangle framework.

#### IS AGROECOLOGY A RELEVANT AND ACHIEVABLE PATHWAY FOR SUSTAINABLE FOOD SYSTEMS?

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Developed by Miguel Altieri in the eighties, the concept of agroecology was recently put forward as a promising pathway for the future of agricultural systems. Agroecology is not only the application of ecological concepts to agricultural practices; it is a new way of designing the food systems with a new vision of the agency of actors. Indeed, prospective exercises such as Agrimonde demonstrated the need for a joint action on both production and consumption of food. The food systems conceptual framework is efficient to discuss issues such as production, access to food, resilience and vulnerability. Moreover, analyses of the present state of agricultural and food systems emphasize the importance of lock-ins and path dependency mechanisms in the persistence of sub-optimal agricultural systems.

Various case studies on pesticide management in potato and dairy systems in Belgium or banana-based systems in Africa will be presented. The main conclusions of the studies are: a) a problem based approach is more prone to the identification of long term and relevant innovations than a solution based approach, b) systemic approaches are easing the appropriation of technical innovations and supporting long term redesign of systems when required, c) articulation of short term efficient technical solutions and long term redesign for building self-sufficient food systems may be cumbersome, d) most of the solutions developed at the plot or plant level will require a reconfiguration of the value chain (transformation, consumers).

The principles of agroecology provide a framework to think and assess innovation. Their application requires new research investment and a better integration of actor and scientist knowledge's. Implementations of agroecology today are mainly based on technical practices (biological control, soil fertility management, agroforestry), sometimes combined. Most of the examples of full redesign of systems are experimental or limited to very specific conditions. Organic agriculture is a long-term alternative pathway where many technical solutions where developed.

Agroecology is a new scientific approach of agricultural and food systems. It offers a serious option to simultaneously address emerging ecological and social challenges. Combination of the agroecological framework with the theory of transition may pave the way for new agricultural systems and open new avenues for transdisciplinary research.

A version of this summary will full references is available at: http://www.philagri.net/?p=1949

**SESSION I** 

# **NEW MANAGEMENT APPROACHES**

#### USING A FLEET OF ROBOTS FOR PATCH SPRAYING

# <u>Fernandez-Quintanilla C.</u><sup>1</sup>, Dorado J.<sup>1</sup>, Ribeiro A.<sup>2</sup>, López-Granados F.<sup>3</sup>, Rabatel G.<sup>4</sup>, Pérez-Ruiz M.<sup>5</sup>, Gonzalez de Santos P.<sup>2</sup>

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Advanced technologies are essential for safe and efficient weed control. Using autonomous mobile platforms (both, ground and aerial) equipped with innovative perception techniques, data processing systems and tools for actuation makes possible to detect and control weeds site-specifically, reducing costs, environmental damages and risks for farmers.

From 2011 to 2014 the RHEA Project, funded by the EU 7<sup>th</sup> Framework Programme, has been involved in the configuration of a new generation of robotic systems for both chemical and physical management of weeds. This article summarizes the RHEA fleet of robots and analyzes the results achieved on the specific case of patch spraying winter wheat crops.

The project has tackled two kinds of missions: (1) an inspection mission, made by aerial units which carry the remote perception system; and (2) a treatment mission performed by ground units equipped with the spraying system. The aerial units were hex-rotor Unmanned Aerial Vehicles (UAVs or "drones") equipped with two Sigma DP2 Merril still cameras. In one of these cameras the NIR blocking filter was removed in order to turn it into a NIR camera. This solution was able to supply a high image quality (4704x3136 pixels) with low-price equipment. The ground mobile units (GMUs) were based on three Boomer-3050 tractors (CNHI) tuned to host the onboard computer, the communication equipment, the GPS (RTK) receiver and the GMU controller. Other elements integrated into the vehicles were a camera system, a laser and GPS and communication antennas. The GMU was carrying a sprayer with a 200L water tank and a 50L herbicide tank connected to a direct injection system to inject the chemicals into 12 solenoid nozzles activated independently and located on a 6m boom. The whole operation of the fleet (three GMUs and two UAVs) was controlled from an external Base Station equipped with a Mission Manager system and communication systems.

In January and May 2014 two assessment trials were conducted in a CSIC Experimental Farm located close to Madrid (Spain), using two artificially weed infested winter wheat fields. The assessments included the evaluation of the precision of the weed map, spraying operation, GMUs trajectories and fleet integration. Around a 95% of the weed patches were detected and over a 97% of them were accurately sprayed with herbicides. Slight delays in the opening and closing of the nozzles and some border errors were observed. The trajectory followed by the ground units barely deviated from the defined route plan pointing out high accuracy, deviating from the planned route less than 7cm (i.e., mean deviation) in the two tests performed. Regarding the performance of the fleet integration, the results showed that all three GMUs were able to work together cooperatively following pre-defined route plans.

#### SENSOR-BASED INTER- AND INTRAROW WEED HOEING

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An autononuous system for online-weed hoeing has been developed for mechanical weed control in soybean, sugarbeet, maize and cereals. The system consists of two completely autonomuous camera-guided hoeing technologies, one for interrow weeding and one for intrarow weeding. Interrow weeding is controlled by a camera system identifying crop rows and steering the interrow hoe close to the crop row. Weed control in the interrow area was performed by duck foot blades.

The system was tested in sugar beet and soybean at driving speeds of 4, 7 and 10 km h<sup>-1</sup>. Between 85% and 89% of all weeds were removed and no crop damage was measured regardless of speed. Intrarow weeding is controlled by a multispectral camera identifying weeds and crops based on shape features. An image analysis software coupled with a micro-controller classifies plants in the image as "annual broad-leafed weed" "grass-weed", "perennial weed" and "crop" and determines the exact position of all plants in relation to the hoe. The camera and the intrarow hoe are automatically triggered by a rotary pulse generator.

The system has so far been trained for weed identification and weed control in sugar beet, soybean, maize and cereal crops. A specific database of plant shapes was developed for each crop. Thresholds were programmed for each plant class to decide if the hoe is turned on or off. In the intrarow area, a modified finger weeder is used for selective weed control. The speed of the finger-hoe is increased at locations where weeds have been identified. The finger weeder is turned only at driving speed at locations where crops or no weeds were classified.

#### PRE-HARVEST ASSESSMENT OF PERENNIAL WEEDS IN CEREALS BASED ON IMAGES FROM UNMANNED AERIAL SYSTEMS (UAS)

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Unmanned aerial systems (UAS) are able to deliver images of agricultural fields of high spatial and temporal resolution. It is, however, not trivial to extract quantitative information about weed infestations from images. This study contributes to weed research by using state-of-the-art computer vision techniques to assess pre-harvest weed infestations in cereals based on true color (RGB) images from consumer grade cameras mounted on UAS. The objective is to develop a fully automatic algorithm in an open programming language, Python, to discriminate and quantify perennial weed infestations in cereals before harvest. Results are compared with an in-house image analysis procedure developed in the commercial eCognition Developer software. The importance of flight altitude and robustness across fields are emphasised.

Image acquisition took place during the summer of 2013 and 2014 in a number of fields under different lighting, weather and growth conditions in spring and winter cereals (barley, wheat and oats). Images were acquired in different altitudes in the range of 10 to 50 m to give different image resolutions (3 to 15 mm/pixel). There were perennial weeds in all fields with *Cirsium arvense* (L.) as the most frequent species.

In order to provide ground truth prior to the modeling phase in Python, a subset of 600 images was annotated by experts with 16000 regions of weeds or crop. Following this, images were segmented into regions with weeds or crop by subdividing each image into 64 by 64 pixel patches and classifying each patch as either crop or weed. A collection of geo-referenced segmented images may subsequently be used to map weed occurrences in fields. To find a robust and fully automated assessment method both texture and color information was used to build a number of different competing weed-crop classifiers, including several variants of the excess green (2G-R-B) vegetation index, and normalizations. The performance of these was measured in terms of classification accuracy. Models were trained offline on the annotated ground truth data (not used for testing). In particular for the texture-based methods, this training is necessary to learn the statistical properties of filter responses from weed and crop patches.

Results emphasise the importance of a broad training context. If models were trained and tested on images representing a narrow range of color and illumination variations, it was possible to achieve more than 95% accuracy, which fully satisfies practical mapping requirements. However, if models were evaluated on images from fields not included in training data, results were varying and unreliable in some fields. In general, the automated image analysis procedure based on color was not competitive with results achieved with eCognition, which provided accuracies in the range of 86% to 92%. Flight altitude and image resolution were not important for the accuracy and ortho-mosaicking had no clear impact. Models including texture-based methods were not fully evaluated because they required hours of computer time per image, and it is doubtful whether their performance can justify the computational expenses. Results are discussed in a practical context and the consequences of varying accuracies are evaluated in different scenarios.

#### AERIAL IMAGERY FOR SITE SPECIFIC WEED MANAGEMENT

#### Louargant M.<sup>1,2</sup>, Vigneau N.<sup>2</sup>., Jones G.<sup>1</sup>, Villette S.<sup>1</sup>, Gée C.<sup>1</sup>

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In order to reduce herbicide application, it becomes crucial to develop new technologies allowing a better knowledge of the field. Thus, proposing a weed localization map may help farmers to manage their fields, improving weed control and reducing input costs and environmental damages. This work aims to develop a new method to localize weeds, by analyzing images acquired with a UAV (Unmanned Aerial Vehicle). The study is carried out on crops widely grown in France (maize, sunflower, sugar beet) and requiring weed control at the early stage of growth.

With its on-board sensors (GPS, multispectral camera...), the UAV can fly over the field to capture a set of images in four spectral bands (green, red, red edge zone and infra-red) with a high spatial resolution. After some image processing and gathering, an orthoimage of the whole field is built.

Two types of information are used to discriminate crops and weeds from the image. First, soil and vegetation (crop and weed) are discriminated according to their reflectance and a vegetation index. Then, the spatial distribution of the vegetation is used to localize weeds. Considering crop rows are straight lines, they are detected using an algorithm based on the Hough Transform.

Weeds are then deduced as the vegetation located outside the rows and infestation maps are drawn, displaying exact weed localization. This method, tested and compared to validation maps (based on ground observations), shows good results for detecting weeds in the inter-row space, but is not able to detect weeds into the rows.

Consequently, to complete this method, the spectral information is explored studying the reflectance of crops and weeds in multispectral aerial images. Thus, the image acquisition chain is modeled in order to optimize the choice of the filters of the camera for crop and weed discrimination in field condition. Then, using statistical analysis, pixels of the image may be classified according to their values of reflectance.

After combining both methods, weeds may be localized in the whole field. Then, with the exact weed localization, a site specific management may be implemented (i.e. precision spraying), reducing the amount of herbicide used per hectare.

#### SMALL WEEDING TOOLS FOR SMALL VEGETABLE FARMS

#### Leblanc M.L.<sup>1</sup>, Lefebvre M.<sup>1</sup>, Moreau G.<sup>1</sup>, and Gilbert P.-A.<sup>2</sup>

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Hand weeding is the main method of weed control for small organic vegetable farms. Hand weeding and hoeing are costly and, in some area such as in Eastern Canada, there is insufficient labour available. Small-scale farmers need new tools that are scaled to the size of their operations and that can decrease the time required to manually remove weeds. Such tools should be versatile and can be used on a multitude of vegetable crops growing on small acreage.

The Weed Master is a versatile manually-pushed tool carrier for seeding, flaming, and hoeing. It is designed to be used in vegetable fields that are up to 2 ha in area. Various weed control equipment can be attached on the same frame including: disk hillers, finger weeders, sweeps and a flame weeder. The machine manufactured in Finland was tested in lettuce, cabbage and carrot for two years. The project was established at the Organic Agriculture Innovation Platform, QC. The objective was to determine Weed Master's efficacy against weeds and how it shortened hand weeding requirements. The experimental design was a randomized complete block design with four replications. The treatments were: hand weeding, Weed master, Weed master + hand weeding, and weedy check.

The best treatment was a combination of Weed Master and hand weeding which improved yield and decreased the time required to hand remove weeds by 46, 38, and 52 % in lettuce, cabbage, and carrot, respectively. In conclusion, effective weed control on small vegetable farms is possible through the use of cultivation and flaming with the Weed Master. The use of the Weed Master can be economically viable because it is faster than hand weeding and provide similar yield.

#### EYEWEED: MAPPING OF BLACK-GRASS (*ALOPECURUS MYOSUROIDES*) INFESTATIONS USING GROUND-BASED MACHINE VISION

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Weeds such as black-grass (*Alopecurus myosuroides* L.) typically occur in patches in arable fields. Farmers, however, will normally spray whole fields even though modern sprayers could confine spraying to the patches. In spite of environmental and economic benefits, adoption of patch spraying by arable farmers has, therefore, been negligible. Reasons for low adoption include the non-availability of geo-referenced, quantitative maps of weed density. This paper describes eyeWeed, a machine vision system, which can be mounted on farm machinery such as sprayers, for automated weed mapping.

Research hypotheses in the eyeWeed project include (1) cameras mounted on ground-based vehicles are needed to capture images of sufficient quality to create farmer-acceptable herbicide application maps, (2) images required for mapping can be captured as an adjunct to normal farming operations such as crop spraying, (3) images can be processed in real time to avoid excessive data storage and (4) classification algorithms are sufficient to detect individual black-grass heads in the field and so obviate the need for ground-truthing. Although the goal is to achieve real-time detection, the further step of real-time detection and control is not proposed since images will normally be captured at times when control is not planned. Moreover the intervention strategies may not simply be post-emergence control but also planning of pre-emergence control and use of higher seed rates in weed patches. A unique feature in comparison to the use of aerial image capture is the avoidance of the need for ground-truthing due to the use of high resolution images.

A further prerequisite is, therefore, that black-grass patches are sufficiently stable so that maps of black-grass heads can be used to predict patch locations of seedlings for pre- and postemergence spraying in the following crop. Because image capture can continue into June or July, long after usual spraying times, maps can be created off-line and may also be used to plan herbicide resistance strategies.

Results are presented describing the system and showing the correlation of manual assessments of manually obtained maps with those derived from the machine vision system. These are linked further with soil maps which may help to enhance the robustness of patch predictions. Some illustrative predictions of the economics and environmental benefits of automating weed mapping and patch spraying will be shown.

Co-funding by Innovate UK is gratefully acknowledged.

#### MANUAL FOR PROPANE-FUELED FLAME WEEDING IN CORN, SOYBEAN, AND SUNFLOWER

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Flame weeding is an approved method for weed control in organic cropping systems, with the potential for use in conventional agriculture. From 2006-2012 we have conducted a series of over 40 studies, which were funded by PERC and other sources (eg. USDA). This extensive work resulted in over 20 journal and proceeding articles about crop tolerance to heat and weed control with flame weeding in field corn, popcorn, sweet corn, sunflower, soybean, sorghum and winter wheat. We compiled the above research information into a training manual that describes the proper use of propane fueled flaming as a weed control tool in six agronomic crops (field corn, popcorn, sweet corn, soybean, sorghum, and sunflower).

Flame weeding manual contains 32 pages of text and color pictures. The pictures provide visuals of crop growth stages when flaming can be conducted safely without having side-effects on crop yield. Pictures of weeds provide visuals of appropriate growth stages when weeds need to be flamed to achieve good weed control. There are six chapters in the manual: (1) The need for alternative weed control methods; (2) Propane fueled-flame weeding; (3) How flame weeding works; (4) Equipment and configurations; (5) Propane dosage at different weed growth stages, and (6) Crop Tolerance to post-emergent flame weeding. We believe that our manual provides a recipe on how to use flaming procedures and it is written in a user friendly manner that can be understood by the general public. Manual is free, it can be downloaded in a pdf format from the following website:

http://www.propane.com/uploadedFiles/Propane/Agriculture/Safety/PropaneFueledFlameWeeding. pdf

#### EFFECTS OF FUNGAL TOXINS ON AMBROSIA ARTEMISIIFOLIA

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Common ragweed, *Ambrosia artemisiifolia* L. (Asteraceae), an annual weed native to Northern America, has become invasive in numerous countries in Europe. South-central and south-eastern European regions are the most affected. In the western and northern parts of Europe and mountain ranges ragweed does not behave as an invasive weed, yet. Climate change is expected to facilitate the establishment of ragweed as a self-propagating weed in these regions in the near future.

The major concern regarding common ragweed is due to its highly allergenic pollen that causes sensitisation of the population, generating huge medical costs. Ragweed also has increasingly become a major weed in European agriculture, especially in spring-sown crops such as sunflower, maize, sugar beet and soya beans. Because of the taxonomic relatedness with sunflower, herbicides are of limited use in this crop, facilitating the spread of *Ambrosia* throughout Europe in birdseed, other feeding mixtures and crop seed.

Herbicides and mechanical control (uprooting, cutting, ploughing) are best suited as local and short-term measures to eradicate initial and small populations, and to mitigate further spread of established populations. In other crops herbicide treatments may be sufficient to prevent yield losses, but often this in not sufficient to prevent ragweed populations from flowering and setting seeds. In non-agricultural land, management of ragweed using herbicides is too expensive and, furthermore, the need to protect the accompanying vegetation does not allow large-scale application of herbicides. Thus, the ragweed population is increasing.

Bioactive fungal metabolites have been long considered for their potential direct use as natural herbicides, as a lead for new herbicides or to discover novel mechanisms of action. The authors of this article have a long history and a strong expertise in the production and chemical and biological characterization of novel bioactive metabolites from microorganisms and plants. However, despite the enormous number of novel metabolites identified and available in their labs, none of them has ever been tested against *A. artemisiifolia*.

Thus, a number of selected metabolites produced and purified by the culture of pathogenic fungi and plants was used in preliminary tests to evaluate their effects on the germination of ragweed seeds, the elongation of seedling rootlets and on the development of necrotic spots on leaves. The present communication reports the first results of these biological assays, discussing the potential use of the most interesting metabolites as natural herbicides for ragweed management.

#### HERBICIDAL EFFICACY OF METABOLITES FROM STREPTOMYCES SCOPULIRIDIS ON UPLAND WEED SPECIES

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This study was conducted to examine herbicidal efficacy of natural substances derived from soil actinomycetes, *Streptomyces scopuliridis*. Herbicidal metabolites extracted from the culture broth of *S. scopuliridis* were mostly consisted of herbicidin A and herbicidin B based on the chemical structure analysis. They inhibited germination of *Echinochloa crusgalli, Digitaria sanguinalis, Euxolus caudatus and Erigeron Canadensis* at concentration of 7.81 ppm in petri dish. Postemergence application of the extract resulted in chlorosis and necrosis at concentration of 2,000 ppm in a greenhouse experiment. Dicot weeds, e.i. *E. caudatus* and *D. sanguinalis* were sensitive to the extract and even low concentration of 3.9 ppm and 62.5 ppm, respectively, inhibited their growth sufficiently. However, the extract was not phytotoxic to the monocot weed species *E. crusgalli*. Physiological and morphological aspects of the activity of this extract should be investigated in order to define the mode of action of these substances in plants.

#### AMINO ACIDS AS OROBANCHICIDES: AN INNOVATIVE APPROACH FOR BIOCONTROL OF BROOMRAPE WEEDS

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Broomrapes (Orobanche and Phelipanche spp) are obligate, chlorophyll-lacking, root parasitic weeds that obtain all their nutrient resources by feeding off many important crops via their haustoria. France suffers from severe infestations of three broomrape species, O. cumana Wallr. infecting sunflower, O. minor Sm. infecting forage legumes and P. ramosa L. (syn O. ramosa) infecting hemp, oilseed rape, and tobacco. Several broomrape traits such as mass seed production, their easy dispersal and longevity, germination induced by host root exudates, specialized host range, and physical and metabolic overlap with the host make the management of these weeds very difficult. Successful control strategies must target parasitic subterranean stages in order to hamper both the main parasitic sink for nutrients and the subsequent parasitic seed production. An effective strategy to control broomrape is the foliar application of systemic herbicides to herbicide-resistant crops. The crop will deliver the herbicidal effect into the broomrape attached to their roots by translocating the herbicide systemically to the young parasite via the haustorium at the host-parasite interface. Among those herbicides, glyphosate, imidazolinones and sulfonylureas specifically inhibit either the aromatic or the branched-chain amino acid synthesis in the parasite by targeting key enzymes in their synthesis pathways. This method is one of the few available strategies efficient for broomrape control however it presents two main drawbacks. First, herbicide resistance is not available for many important crops and second, increasing concerns for environmental pollution. An environmental-friendly alternative to this method could be the use of broomrape-specific inhibitory amino acids. Amino acid synthesis pathways are regulated by feedback inhibition of controlling enzymes by some of the amino acid end-products resulting in starvation of other amino acids in the same pathway and as consequence growth inhibition. The suppressive activity of amino acid end-products varies according with species and life stages. Laboratory experiments revealed that methionine and lysine inhibit the germination and radicle elongation of the broomrape species affecting French agriculture. This developmental inhibition hampers the infective potential of broomrape seedbank in the host crop. Additional mechanisms such as elicitation of resistance by methionine against Orobanche root penetration as well as the use of selected and characterized strains of *Fusarium oxysporum* f. sp. orthoceras are being investigated. Methionine and lysine are produced in large scale as animal feed supplements and can be delivered to the soil during the short infective period in which broomrape germination and host infection occur. The persistence of the amino acid is predicted to be low beyond this short time frame due to soil microbial metabolization. The Orobanche Biocontrol Project at INRA-Dijon is carrying out in vitro, rhizotron and field experiments aimed to optimize the potential use of methionine and lysine as orobanchicides. This project is funded by Santé des Plantes et Environnement division (SPE-www.spe.inra.fr).

#### RGB IMAGERY AS BASIS FOR SITE-SPECIFIC MANAGEMENT OF PERENNIAL WEEDS

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The purpose of our study is to explore the possibility to use proximate RGB imagery as basis for site-specific management of perennial weeds in small grain cereals. The targeted species are the broadleaved weeds *Cirsium arvense* (L.) Scop. (Creeping thistle) and *Sonchus arvensis* L. (Perennial sowthistle) and the grass weed *Elymus repens* (L.) Gould. (Common quackgrass). These are the main challenges for perennial weed control in cereals in Norway and temperate zone.

The overall idea is to make weed maps based on images acquired during harvest in autumn (August/September) and use these maps for site-specific weed management when these species are normally managed in Norway, i.e. 3-4 weeks after harvest (*E. repens*) or in the following spring, i.e. late May/early June (*C. arvense* and *S. arvensis*). An on-the-go weed detection and glyphosate application in one operation before harvest is also a possible usage of our image-based method where this timing of glyphosate application is allowed.

Images were acquired with a consumer grade camera mounted on a 3 m pole and tilted to mimic images acquired from the roof of a combine harvester. Images were acquired few days before harvest, a period where the cereals are yellowish and weed leaves and stalks are still green. Plots, 8 m by 8 m, were established in cereals to cover a wide range in weed pressure- and flora. The four plot corners were marked with white styrofoam balls mounted on sticks prior imaging and recorded with GPS (10 cm accuracy).

The machine vision algorithm performs first a geometrical transform to represent the images as pseudo-orthonormal to the ground plane. This transform is aided by white styrofoam balls marking the corners of the plot with known distance. In the intended practical use, the transform can be done by obtaining the camera-angle and heading from inertial and GPS measurements and assuming level ground.

The classification algorithm starts by segmenting the image into a class for green parts of the weeds (leaf, stalk), and three classes for flower heads (yellow, white and purple), by using threshold filters in the HSV colour space. A connected components analysis is then performed on each of the binary images, where the very small regions are filtered out. The area and centre of each region is calculated for comparison with ground truth observations.

Two types of ground truth data for evaluation of the algorithm are available: Manual assessment of weed coverage from computer display of images and weed maps based on GPS measurements at the time for their management. Machine vision algorithm outputs versus ground truth data will be presented.

#### **PRECISION CONTROL OF WEEDS IN CARROTS AND ONIONS – CHALLENGES**

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Precision control of weeds requires information about the location of the weeds and the crop accurate enough to target the weeds while avoiding the crop. For larger crop plants planted at regular spacing such as cabbages or lettuce, vision technologies taking the array structure of the planting pattern into account where developed and implemented on precision mechanical weeders, allowing both inter and intrarow weeding. For crops such as carrots or onions, planting patterns are more challenging to identify based on vision technologies. For example, when carrots are seeded following a staggered pattern, the main row is split into 3 sub-rows. At the one leaf stage, unless there is heavy weed infestation, the pattern is visible but at the three leaf stage the pattern is almost completely occluded by crop leaves. On the other hand, young onions are very thin when viewed from above and the planting pattern may be hard to define due to their elongated shape. Furthermore, weeds may interfere in the proper identification of the seeding pattern.

Image analysis strategies were developed in order to uncover the seeding pattern of carrots and onions from photographs taken from above the canopy. These strategies will be presented and their use at various crop stages and weed infestation levels will be assessed in terms of accuracy and computational requirements. Clearly, the sooner the better: at the very early stage, seeding patterns are obvious. On the other hand, weeding at very early stages can be challenging. For example, weeding in carrots at early stages has to be done with minimal soil disturbance as the young carrot taproots are easily distorted by any significant soil movement.

For vision based precision weeding to be possible in carrots and onions, appropriate seeding patterns must be selected. This may not be obvious as current seeding patterns evolved from other agronomic requirements and the tools and machinery used on commercial farms where developed to suit these patterns.

#### **BIODEGRADABLE PAPER MULCH FOR WEED CONTROL**

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Weed control with plastic films is a common practice in vegetable, berry and fruit production. Plastic mulch improves both the amount and quality of crop yield and reduces the need for pesticides, fertilizers and irrigation. The disadvantage of plastic mulch is that after harvesting vegetables the polyethylene film becomes waste material that should be removed from field to dump at high cost. Therefore, biodegradable mulches of various materials have been developed and introduced. A recent innovation from Finland is to treat special paper with wood distillate in order to prolong the decomposition of biodegradable paper mulch. Field experiments in vegetables were carried out in 2013-2014 to demonstrate the feasibility and longevity of paper mulch. Both biological and technological aspects have been explored not only in Finland but also in Egypt, Germany, Spain and The Netherlands.

Testing of wood-based liquids for plant protection purposes started in 2003 and mulching technologies have been developed since 2008. MTT Agrifood Research Finland (now "Luke") assigned IPR rights for the wood-vinegar based mulching innovation to Stora Enso Oyj in 2011. Thereafter development of the technology has been continued as a joint R&D project.

Field trials with biodegradable paper mulch have been carried out in cabbage, celeriac, cucumber, onion and zucchini in southern Finland. Preliminary tests with multi-season paper have been established in strawberry. Black polyethylene film and biodegradable starch-based mulch (Finnish product called "Bioska") were used as commercial standards.

The first challenge for paper mulch is mechanical laying on the soil. This phase requires gentle modern machinery that covers the paper margins with soil. The soil has to be tilled properly in order to avoid tearing the paper. The degradation of edges is rapid, particularly if the paper's margins are not treated with sufficient wood distillate. Consequently, wind easily tears the mulch from the ground too early. The size of planting holes played a significant role in terms of weed emergence.

Weed control efficacy was good because the weed seedlings could not emerge either through the paper mulch or plastic film. In contrast, grass weeds, *Cirsium arvense* and *Equisetum arvense* penetrated the starch-based mulch. In general, there was no significant difference in weed control efficacy among the mulches investigated.

The first experiences with biodegradable paper mulch are relatively convincing. The weed control efficacy is excellent as long as the paper remains on the ground and the planting holes are kept to a minimum size. A fast-growing crop like cabbage manages with late-emerging weeds. Still, hand-weeding of weed plants emerging from planting holes is often required. Moreover, the bare soil between mulch-covered crop rows must be kept weed-free by some means. The paper type treated with a sufficient wood distillate is durable enough for annual vegetables. Biodegradable mulches are more expensive than polyethylene film, but removal costs of plastic even out the final costs. Field tests with the Finnish paper mulch types will continue in the 2015 growing season.

#### EXCELLENT WEED CONTROL WITH BIODEGRADABLE MULCHES IN PEPPER

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The most-used weed control technique for pepper grown open-air in Spain is mulching with polyethylene (PE) film. The management of the mulch waste after harvest is costly and time-consuming. Several biodegradable materials are available in the market but no results of comparison are available for pepper. The aim of this work was to test several biodegradable black mulch films and paper for their weed control and yield at four different locations in Spain under different climatic conditions. With this purpose, field trials were established at: Zaragoza (Z), Ciudad Real (CR), Cadreita (CA) and Lleida (L).

The following treatments were tested: 1) PE; biodegradable films: 2) Mater-Bi® (maize starchbased), 3) Sphere 4 (potato starch-based), 4) Sphere 6 (potato starch-based), 5) Bioflex® (polylactic acid-based), 6) Ecovio® (polylactic acid-based), 7) Mimgreen® (paper, 85 g/m<sup>2</sup>); control treatments: 8) unweeded control, 9) manually weeded control. Materials were placed on the soil mechanically and seedlings were planted at the same density with the herringbone method at all locations between May 19<sup>th</sup> and 22<sup>nd</sup> 2014 on 15-25 meter long rows. Each row was considered a treatment and replicates were distributed randomly in three-four blocks depending on the location. Drip irrigation was conducted following water demand of each treatment. Weed density was assessed 21, 42 and 63 days after planting (DAP) in four fixed 0.2 x 1 m frames on the mulched surface. At harvest, commercial and non-commercial fruits were weighed separately for each row and total yield was calculated as a sum.

Weed species composition was different for each location being the main species *Cyperus rotundus*, *Setaria* spp. and *Digitaria sanguinalis* at Z; *Diplotaxis virgata* and *Amaranthus albus* at CR; *Stellaria media* and *Cirsium arvense* at CA; *Portulaca oleracea* and *Amaranthus retroflexus* at LL. Total weed density in the unweeded control plots 63 DAP was 62, 86, 23 and 235 plants m<sup>-2</sup> at Z, CR, CA and L, respectively. Weed control efficacy exceeded 90% for all plastic mulches excepting where *C. rotundus* was found (Z). There, efficacy ranged between 20 and 72% being the highest for Sphere 6 and PE and the lowest for Sphere 4 and Bioflex®, which were the materials mostly pierced by this weed at densities of 7-15 plants m<sup>-2</sup> depending on the treatment. The paper mulch controlled satisfactorily *C. rotundus*.

Both commercial and total yield were significantly higher for the mulched treatments compared to the unweeded plots at all locations. The manually-weeded plots yielded intermediate at two sites. All the tested mulches demonstrated a high weed control capacity excepting for *C. rotundus* and yielded as much as PE at all four sites with different edapho-climatic conditions offering technically viable alternatives to PE.
# **SESSION IIA**

# CHEMICAL WEED MANAGEMENT

#### INVESTIGATING GLYPHOSATE USE AND WEED PATTERNS IN GERMAN ARABLE FARMING

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Glyphosate is worldwide the most sold agricultural chemical. In Germany, the use of glyphosate-based herbicides has increased 100% since the year 2000. According to a study carried out in Germany in 2010/2011, glyphosate-based herbicides are applied on nearly 40% of the total arable area; thus, glyphosate is an important component of vegetation management in German arable farming. There are three major uses of glyphosate products in German arable farming: stubble (68.1%), pre-sowing (20.7%) and pre-harvest application (11.2%).

Studies that further quantify the use of glyphosate and relate it to agronomic practice are rare. Since glyphosate was introduced to the market in 1974, only three major studies have been published that analyses the use of glyphosate products in the United Kingdom and Germany. Studies that measure the influence of farm production conditions on glyphosate use intensity are missing completely.

Globally reports about arable weed species that developed resistance against glyphosate are increasing. A strong relation to glyphosate resistant (GMO) crops is obvious, although spontaneous resistance in weeds is not exclusively occurring in these cropping systems. In Germany these varieties of crops are not registered or produced. Nevertheless the use of glyphosate in arable cropping is frequent. While no glyphosate resistant weeds were found in Germany so far, we hypothesize reactions of the weed vegetation on a species level.

We handle both aspects in a project which aims on identifying Best-Management-Practice for including glyphosate products in cropping systems. While the use is nation-wide surveyed among German farmers in 2014/2015, the reaction of the weed vegetation is field-wise monitored in North-East Germany. 2016 farmers participated in the survey and sent us data about their usage of glyphosate products in arable cropping. Furthermore they replied on questions about the relevance of glyphosate for their cropping systems. First analyses of the data will be presented. The weed survey takes place in 2014 and 2015 on approx.. 50 fields each year in Mecklenburg-Vorpommern. Winter wheat fields are sampled due to their proposed frequency of Glyphosate applications during recent years. We use 100 m<sup>2</sup> monitoring plots and take three samples per field.

## PREDICTION OF HERBICIDE EFFICACY IN THE DECISION SUPPORT SYSTEM DSSHERBICIDE

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DSSHerbicide is a decision support system for chemical weed control in winter wheat. It looks for low-cost herbicide solutions with the constraint, that possibly surviving weeds will economically harm neither the winter wheat nor future crops. Costs for chemical weed control can be reduced by choosing an herbicide combination with an efficacy profile that fits with the weed infestation in the field. An additional cost reduction is possible by reduction of the dose. Therefore, good knowledge about dose-response relationships for each weed species – herbicide combination is necessary.

One crucial point in identifying an optimized herbicide solution is the accurate prediction of the herbicide efficacy in the field. Another crucial point is the level of target efficacy. Target efficacies are specified by plant protection experts specifically for weed species, for different weed densities and weed development stages. They are implemented in the DSSHerbicide as the minimum efficacies necessary to prevent yield losses.

DSSHerbicide was tested in sixteen herbicide field trials with three different target herbicide efficacy levels in North-East Germany for two years.

We present target efficacies, predicted herbicide efficacies and measured herbicide efficacies from autumn sprayings in the fields. Target efficacies, predicted efficacies and measured efficacies will be compared to answer the following research questions:

i) Did DSSHerbicide find herbicide solutions suited for the weed infestations in the field trials?

ii) How exact could DSSHerbicide predict herbicide efficacy in the field?

iii) Did the DSSHerbicide recommendations result in herbicide efficacies acceptable for practical agriculture?

The results will be discussed with focus on weed control and environmental herbicide load.

#### RESPONSE OF GRASS WEEDS TO HERBICIDES DEPENDING ON SPRAYING TECHNIQUES AND SPRAY LIQUID PROPERTIES

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Annual grasses are a major weed problem both in winter and spring agricultural crops. Grass weeds at the optimum growth stage are a difficult spray target with post-emergence herbicides due to their predominantly vertical leaf positioning. This result in low spray deposits on the grass weed plants reducing the biological effect and potentially increasing the risk of herbicide resistance. Formulation of agrochemicals, as well as the adjuvants and diluents that are added to the spray solution before application, can change and influence the spraying process by changing physical properties such as surface tension, viscosity and density.

The objective of the greenhouse, field and laboratory experiments was to determine the influence of spraying techniques (droplet size, spray volume) and liquid properties on the activity of the herbicides fenoxaprop-P-ethyl and propoxycarbazone-sodium against wild oat (*Avena fatua* L.) and blackgrass (*Alopecurus myosuroides* Huds.).

The droplet size pattern of different nozzle design was measured using Malvern laser measuring techniques. Three nozzle types that produce very fine, medium and very coarse spray quality were tested and classified based on droplet size spectra and the British Crop Protection Council (BCPC) classification scheme.

Experiments showed that the use of very small and medium spray quality significantly increased the effectiveness of fenoxaprop-P-ethyl against difficult-to-wet weed species like wild oat and blackgrass, especially when the herbicides were applied at the 1-2 leaf stage. Air induction nozzle producing bigger droplets resulted in a significant decreased retention and herbicidal activity. Spray volumes of 125 and 250 I/ha resulted in effective control of the grass weed species by fenoxaprop-P-ethyl and propoxycarbazone-sodium.

Droplet size was the most important spray application parameter influencing herbicides effectiveness against grass weeds. For a given spray volume, smaller droplets increased coverage of target area, retention of droplets and efficacy. Impact of droplet size was most pronounced when herbicides were applied at early growth stages. Air induction nozzles producing coarse and very coarse spray quality led to a significantly decreased herbicidal effect. The adjuvant Torpedo II increased the herbicidal effect, but the effect depended on herbicide type and formulation and as well as on the growth stage of target species. Addition of adjuvant to the spray mitigated or masked the differences in efficacy between nozzles type and spray volumes. In summary, the results revealed that lowering the spray volume increased the efficiency against grass weeds of foliage-applied herbicides.

#### ASPECTS OF GLYPHOSATE MECHANISM IN EGYPTIAN BROOMRAPE CONTROL

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*Phelipanche aegyptiaca* (Egyptian broomrape) is an obligate holoparasite belonging to the Orobanchaceae. The parasite attaches to the root of the host plant and invades its vascular system using a complex organ called haustorium. *P. aegyptiaca* tubercles function as a strong sink and draw all of their nutritional requirements from the host plant through the haustorium. Glyphosate is a non-selective herbicide that inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimate pathway and in aromatic amino acids (AAA) biosynthesis. *P. aegyptiaca* is efficiently controlled by glyphosate when applied on the foliage of a tolerant host plant. The general notion suggests that glyphosate controls plants by causing AAA deficiency. However, since the parasite receives all of its nutritional requirements from the host, the mechanism for its control is not clear. The objective of the current study is to elucidate the mechanism by which glyphosate controls *P. aegyptiaca*.

In order to distinguish between the effect of glyphosate on the parasite from its effect on the host, glyphosate resistant tomato (GRT) were used as host plants. We have detected high accumulation of shikimate in *P. aegyptiaca* tubercles following glyphosate application. Conversely, the GRT host sink tissues did not contain high levels of shikimate. Shikimate is known to accumulate in response to EPSPS inhibition, thus indicating that *P. aegyptiaca* possesses a functional EPSPS.

Viability of *P. aegyptiaca* tubercle decreased within 40 hours after glyphosate treatment. Quantification of the enzymatic activity and membrane integrity using fluorescein di-acetate revealed that the reduction was site related. Parenchymatic tissues of the haustorium and the tubercle core lost viability during that period while other tissues, such as the parasite apical meristem and vascular supportive tissues were not significantly influenced. In addition, viability loss rate was found to be related to the parasite developmental stage. Tubercles at primary stages of development (i.e. tubercle and rooted tubercle) were more sensitive to the herbicide and exhibited a higher rate of injury, in oppose to later stages of the parasite development, when the apex had already formed.

In order to examine glyphosate effect on solutes translocation, we have crossed the GRT line with a tomato plant expressing GFP under the *AtSUC2* promoter. The hybrid host translocates the fluorescent protein in the phloem and is resistant to glyphosate. *P. aegyptiaca* tubercles exhibited fluorescence once attached to the host root vascular system. Quantifying the GFP fluorescence intensity revealed that the fluorescence intensified during the early period of parasitism. However, soon after glyphosate application fluorescence levels gradually decreased and eventually disappeared. These findings imply that glyphosate weakened the sink strength of the parasite and actually prevented its supply of assimilates.

We hypothesize that glyphosate disrupts carbon metabolism in the parasite by inhibiting EPSPS, weakening the parasite sink strength and therefore interrupts with solutes transport that eventually leads to its death.

#### ASSESSMENT OF DRIFT POTENTIAL OF SPRAYS PRODUCED FROM FORWARD TILTED SHIELDED ROTARY ATOMIZERS COMPARED TO HYDRAULIC NOZZLES

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Crop protection is mainly achieved by applying Plant Protection Products (PPP) using hydraulic nozzles, which rely on pressure, to produce a wide droplet size distribution. Because of increasing concerns about drift reduction, a wide range of low drift nozzles, such as air induction nozzles, was introduced in order to reduce the finest part of the spray. While successful for some treatments, the efficiency of coarser sprays is dramatically reduced on small and superhydrophobic target surface, i.e. at early stage weed control. This may be related to the increased proportion of big bouncing and splashing droplets. On the other hand, Controlled Droplet Application (CDA), using shielded rotary atomizers, stands for an improved control of droplets diameters and trajectories compared to hydraulic nozzles. Unfortunately, these atomizers, because of their horizontal droplet release, are widely recognized to produce more drift than hydraulic nozzles. The present study investigated whether the setting of a rotary atomizer 60° forward tilted can reduce drift to acceptable levels in comparison with vertical and 60° forward tilted standard and low drift flat fan nozzles for the same flow rate.

In a wind tunnel, the drift potential of a medium spray produced by a tilted shielded rotary atomizer Micromax 120 was benchmarked with that of a flat fan nozzle XR11002 fine spray and that of an anti-drift nozzle Hardi Injet 015 medium spray. Operating parameters were set to apply 0.56 l/min for all spray configurations. Vertical drift profiles were measured 2.0 m downward from nozzle axis for a 2 m.s<sup>-1</sup> wind speed. The tilted hydraulic nozzles resulted in a significant drift increase while droplets trajectories are affected by the decrease of the droplet initial vertical speed. Droplets emitted by the shielded rotary atomizer drifted due to low entrained air and turbulence. A significant reduction of the cumulative drift was achieved by the rotary atomizer in comparison with flat fan nozzle while still being higher than the anti-drift nozzle. Unfortunately, the drift potential index (DIX) revealed that the cumulative drift reduction may not results in actual drift decrease because of higher drift at higher sampling locations. As a result, the DIX of the shielded rotary atomizer was similar to the standard flat-fan nozzle while the anti drift nozzle, as expected, reduced drift drastically. Therefore, the 60° tilted rotary atomizer failed to reach low drift levels as expected despite the reduced span.

Keywords: Tilted shielded rotary atomiser, hydraulic nozzles, drift potential, wind tunnel.

#### CHARACTERIZATION OF AMBROSIA ARTEMISIIFOLIA DOSE-RESPONSE TO ALS-INHIBITING HERBICIDES

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Common ragweed (*Ambrosia artemisiifolia, Asteraceae*) is an annual herb mostly known in Europe as an invasive plant originating from North America. Ragweed colonizes different types of habitats (railways, river sides, wastelands, farmlands...) including cultivated fields. Resistance to linuron, glyphosate and/or acetolactate synthase (ALS) inhibitors has been reported in this species in North America, which hampers its chemical control. *A. artemisiifolia* is also a troublesome weed in Europe. Because ALS inhibitors are currently among the most effective tool to control this species in cultivated fields, the recent commercialisation of ALS inhibitor-tolerant sunflower varieties has allowed growers to achieve satisfactory chemical control of common ragweed. However, increased use of ALS inhibitors against *A. artemisiifolia* raises the question of the risk for selection of resistance to these herbicides. Our purpose was to gather reference sensitivity data and tools to be able to assess the risk for resistance evolution in *A. artemisiifolia*.

A PCR-based assay was developed to detect mutations at ALS codons crucial for herbicide sensitivity in A. artemisiifolia. Baseline sensitivity data was obtained for six broadly and frequently used ALS inhibitors (imidazolinones: imazamox, sulfonylureas: metsulfuron, tribenuron, tritosulfuron, prosulfuron and triazolopyrimidines: florasulam) from herbicide dose-response assays performed on two French A. artemisiifolia field populations (P08 and P14). Herbicide doseresponse curves were established on the basis of plant dry biomasses. GR50 and MIC (GR100) values defined as the herbicide rates reducing plant biomass by 50% and 100%, respectively, compared to the untreated reference, were estimated. Differences in herbicide response were observed between populations and among herbicides, with GR50 values ranging from 0.02N (with metsulfuron or prosulfuron) to 0.1N (with imazamox) for population P08, and from 0.03N (with prosulfuron) to 0.42N (with metsulfuron) for population P14, N being the maximum registered field rate. The MIC values were higher than the recommended field rates for three sulfonylurea herbicides (metsulfuron, tribenuron and prosulfuron) for both populations. Two other herbicides (tritosulfuron and imazamox) were characterized by MIC values lower than the recommended field rates for both populations. Florasulam showed a low MIC value for P08 and a higher one for P14 (0.42N vs. 1.16N respectively).

Overall, plants in population P14 were less sensitive to the herbicides assayed and displayed higher variation in biomass at each herbicide rate used, but ALS genotyping of the least sensitive plants did not identify mutant ALS alleles. The variation in sensitivity observed among *A. artemisiifolia* populations and plants suggests there is potential for the evolution of non-target-site based resistance to ALS inhibitors in this species.

#### ARE HERBICIDE EFFICACY, SELECTIVITY AND RESISTANCE FACTORS WELL DEFINED BY DOSE-RESPONSE CURVES?

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Herbicides are designed to kill plants. Sufficiently high doses kill both crops and weeds, whilst small doses have no or little effect. For the selective herbicides the dose-range windows, which control weeds without harming the crop too much, must be quantified in dose ranges. The action of a herbicide is usually determined by its chemical and physical properties, its effect on plant metabolism, the stage of development of the plant and the environment, and last but not least the concentration of the active ingredients at the target site. That said a dose-response curve gives a snapshot of an otherwise dynamic process.

The objective of this abstract is to give an overview of the basic principles of how to quantitatively assess herbicide efficacy, selectivity and resistance factors. Although weed science is rooted in agronomy the best way to assess herbicide selectivity is to use the principles of toxicology that classify xenobiotics according to their toxic profile; the first of which is the acute toxicity linked to the dose required to kill 50% ( $LD_{50}$ ) of some test organisms (e.g., rats, mice). However, the principles in weed science have focus not only on general toxicity, but also on selectivity and resistant factors.

Selectivity of a herbicide can be defined by comparing several dose-response curves of weeds and crops simultaneously and quantify the findings in relative terms. We must define a standard herbicide and a test herbicide and estimate a relative potency, which at  $ED_{50}$  is the distance between two dose-response curves. Some weed scientists would argue that  $ED_{10}$ , crop tolerance, and  $ED_{90}$ , weed control, would be much better parameters to assess selectivity. We agree, but  $ED_{50}$  is the most precise parameters of a sigmoid dose-response curve.

In toxicology pesticides are classified into toxic categories on the basis of  $LD_{50}$ . This makes it easy to compare compounds because of the binomial response, dead or alive, and the responses usually span between 0 and 1.

In weed science most published papers use for example biomass of no pre-defined upper limit in the non-treated control. The problem of differential response in non-treated controls must be addressed, although it is often 'side-stepped' by rescaling the response. Transforming continuous data to percent of a non-treated control for each test plant forfeits important information about the growth conditions of the test plants.

Efficacy, selectivity and resistance factor estimates on the basis of biomass, height etc should be done on raw data to tell the end user of the research how the growth conditions were and how large the differences were in non-treated controls. The tools for applying these principles will be illustrated, based on the discussion at a new website at URL: <u>http://rstats4ag.org/</u>

#### CHEMICAL CONTROL OF SENSITIVE AND RESISTANT TO ALS INHIBITORS APERA SPICA-VENTI POPULATIONS

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Silky bent grass (*Apera spica-venti*) is an annual winter weed native to Europe and North Asia. This grass species causes severe problems in Poland and neighbouring countries, especially in winter wheat. In practice silky bent grass control relies mainly on chemical control with predominance of ALS inhibitors. This factor together with poor rotation of chemicals with different mode of action result in selection of resistance to herbicides from this group.

The aim of this work was to assess the level of control of *A. spica-venti* populations by different herbicides and their mixtures used to control weeds in winter wheat (first year of the study) and in rape (second year of the study).

In the study three populations of *A. spica-venti* were used: one sensitive and two resistant to ALS inhibitors Ten plants per population were planted in separate pots in the greenhouse. Six combinations of herbicides dedicated for weed control in winter wheat were applied at 2-3 leaf stage of *A. spica-venti* at field rates. Plants which survived herbicide treatment were cultivated under isolation to prevent pollination between different populations and treatments. Seeds produced by survivors were sown to the pots where maternal plants had been cultivated. Subsequently silky bent grass seedlings at 2-3 leaf stage were treated with herbicides recommended for use in rape at field rates. In each year of the study after herbicide treatment level of *A. spica-venti* control was assessed. In the first year study the best control of all silky bent grass populations was achieved with chlorsulfuron+isoproturon (100% control). In the second year ethametsulfuron+metazachlor and ethametsulfuron+quizalophop-p-ethyl showed to be the most effective with the level of control 97 – 100%.

#### RESISTANCE MANAGEMENT AND CONTROL OF SULFONYLUREA-RESISTANT BROADLEAF WEEDS IN CEREALS WITH THE PHENOXYALKANOATES, PICOLINATE, PYRIDYLOXYALKANOATE AND ARYLPICOLINATE HERBICIDES BELONGING TO GROUP O (AUXINIC HERBICIDES)

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A total of 14 auxinic (HRAC group O) herbicides are currently registered in the E.U. for controlling weed species in crops such as cereals. Many of these herbicides have been on the European market for decades (2,4-D and fluroxypyr for more than 50 and 30 years respectively). Despite the length of time these herbicides have been used, resistance to this class of herbicide is still low when compared to the high risk groups such as the ALS herbicides (group B), which have been on the market for 30 years.

In European cereals, two annual broad leaf weed species have developed resistance to auxinic herbicides: *Stellaria media* to mecoprop in the UK, and *Papaver rhoeas* to 2,4-D in Spain and Italy, with 1 and 3 confirmed cases, respectively. To date there are no confirmed cases of resistance to the pyridine carboxylic acid herbicides in Europe. In comparison, at least 7 annual broad leaf weed species (*Matricaria species, Papaver rhoeas, Stellaria media, Sinapis arvensis, Galeopsis tetrahit, Capsella bursa-pastoris,* and *Centaurea cyanus*) have developed resistance to the ALS herbicides in multiple locations. The continued and successful use of the group O herbicides in European cereals further supports its low risk status.

Within the group O herbicides, there are a number of structurally distinct classes which include: phenoxyalkanoates (e.g., 2, 4-D and MCPA); picolinates (aminopyralid, clopyralid and picloram); pyridyloxyalkanoates (triclopyr and fluroxypyr); arylpicolinates (Arylex<sup>™</sup> active); benzoates (dicamba), and quinoline carboxylates (quinclorac).

After more than thirty years of use in European agriculture phenoxyalkanoates, picolinate and pyridyloxyalkanoate herbicides continue to provide reliable control of economically important broadleaf weed species in cereals. The arylpicolinate herbicide, Arylex, provides yet another group O herbicide that further contributes to broad spectrum broadleaf weed control in cereal crops. These herbicides are also important resistant management tools for the control of sulfonylurea-resistant species: *Matricaria species* with clopyralid, *P. rhoeas* with aminopyralid and Arylex; *S. media* with fluroxypyr, *C. cyanus* with clopyralid and aminopyralid and *G. tetrahit* with fluroxypyr and Arylex.

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#### HERBICIDE CONTROL OF IMIDAZOLINONE RESISTANT VOLUNTEER OIL SEED RAPE IN WINTER WHEAT

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The introduction of herbicide tolerant (HT) technologies lead to significant progress of weed control in arable crops, where the use of herbicide were limited and weed management was difficult. Using HT hybrid oilseed rape (OSR) (*Brassica napus*) has agronomic and environmental benefits, but its use can result in problems with volunteer plants is succeeding crops.

In 2013, a small plot experiment in winter wheat was established on locations with different climatic and soil conditions. On one half of the experimental area IMI volunteer OSR was sown, on the other half of the experimental area conventional OSR was sown. Six different herbicides were applied in the experiment. The application was made at two application timings (T1: BBCH 11 - autumn, T2: BBCH 27 - spring). The efficacy of the herbicides against imidazolinone-tolerant (IMI) and conventional volunteer plants of OSR as well as winter wheat yield and quality were evaluated.

IMI volunteer OSR was successfully controlled with autumn applications of herbicides containing combination of PDS inhibitors with oxyacetamides (diflufenican, flufenacet) or herbicide combining PDS inhibitors with triazolopyrimedines (diflufenican, florasulam, penoxsulam). Herbicides containing combinations of synthetic auxins with triazolopyrimedines (2.4-D, aminopyralid, florasulam) ensured very good efficacy of spring application. Concerning timing of application it was found that autumn application increased yield by approximately 10%. The autumn application ensured early OSR volunteer suppression and it was positively reflected in yield.

#### HERBICIDE CONTROL OF CIRSIUM ARVENSE IN WINTER WHEAT, CZECH REPUBLIC

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Creeping thistle *Cirsium arvense* (L.) Scop. is classified as a very dangerous perennial weed and it ranks among 10 most harmful weeds of the world. Its competitive ability in wheat is high. Control of creeping thistle is possible by using several methods of weed management: rotation of cultivated plants, mechanical and especially herbicide control.

Four herbicides were applied at three growth stages of creeping thistle in a small-plot experiment for 3 years (2011-2013). The efficacy of chosen herbicides on creeping thistle and their effects on root system, regeneration of thistle plants and its regrowth after winter wheat harvest were evaluated visually. Additionally, the yield and quality parameters of winter wheat were evaluated.

Creeping thistle was successfully controlled with herbicides containing combination of synthetic auxins and ALS inhibitors. The most considerable efficacy was obtained at first application timing. Applications of herbicides at later growth stages of creeping thistle led to its earlier regeneration. Roots of all herbicide treated variants showed symptoms of damage, colour change, reduction in root hairs and the diameter of primary root. The most intensive regrowth of creeping thistle after harvest of winter wheat was observed for herbicides containing syntetic auxins.

#### AN OPTIMIZED GLYPHOSATE FORMULATION

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Glyphosate efficacy is affected by many environmental, biological and application factors, including low air temperature and humidity, presence of hard-to-control weed species, advanced weed growth stage, high level of antagonistic ions in the spray water and/or high application volumes. Activator adjuvants play a key role in the delivery of herbicide active ingredients to the target. However, single-component activator adjuvants incorporated into traditional glyphosate formulations by manufactures or added into the spray mixture by farmers rarely affect all important factors responsible for efficacy. Thus, to optimize glyphosate performance under a wide range of environmental, application and biological conditions, laboratory and field experiments were conducted to search for synergistic and/or additive interactions of different adjuvants and incorporate the most effective compounds directly into herbicide formulation. As a result of this research a new glyphosate formulation was invented that contain multifunctional adjuvant mixture comprising non-ionic surfactants, ammonium salts, organic acid, pH buffer and humectant. Biological efficacy of this formulation was tested against Elymus repens, Lolium perenne and Brassica napus in the field in three locations and conventional glyphosate 360 SL formulation containing a single surfactant was included as a reference. Physical properties of spray solution (surface tension and spray droplet contact angle), pH, chlorophyll fluorescence and C-14 glyphosate absorption were measured in laboratory and greenhouse experiments. Hard water containing 300 mg/L of calcium ions was used in all experiments as a herbicide carrier. Laboratory and field experiments were conducted at Poznan University of Life Sciences and SN Biotech Technologies, Poland. Glyphosate treated plant fluorescence and glyphosate absorption experiments were commissioned to SURfaPLUS R&D, Wageningen, Netherlands.

The new glyphosate formulation containing build-in multifunctional adjuvant greatly reduced static and dynamic surface tension of spray droplets and provided better surface wetting then the reference glyphosate 360 SL formulation. Also results from C-14 glyphosate absorption and fluorescence experiments indicate that this formulation was more efficacious by providing more active ingredient to the target than the reference glyphosate formulation containing single component adjuvant. Biological performance of the new glyphosate formulation in the field experiments was significantly higher, regardless of bioassay plant species and location. Efficacy evaluations made 2, 4 and 8 weeks after treatment and *Elymus repens* regrowth evaluation 6 month after application indicate that by applying glyphosate formulation with build-in multifunctional adjuvant the rate of herbicide active ingredient may be reduced up to 50%, without significant reduction of efficacy.

#### EFFICACY OF RICE HERBICIDES ON ECHINOCHLOA SPP. AS AFFECTED BY REPEATED USE

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Successful control of species belonging to the genus *Echinochloa* (P. Beauv.) in Italian rice fields has become increasingly difficult because of resistance to herbicides currently available for use in rice.

The objective of the study was to compare under Italian field conditions the evolution in efficacy on *Echinochloa* spp. of ALS-inhibitor and ACCase-inhibitor herbicides for three growing seasons (2010-2012). Penoxsulam, cyhalofop-butyl, and profoxydym applied at different rates, either alone, in combination, or in a sequential application. The study was divided into three different trials: trial 1 started in 2010 and lasted for three years; trial 2 started in 2011 with a two year duration; and trial 3 was carried out for a one year duration in 2012. For trials 1 and 2, treatments were applied to the same plots each year. Treatments were arranged in a RCBD with four replications (plot size 10 x 5.5 m). Before the study (2006-2009 seasons), profoxydim and penoxsulam were used in alternate years in the experimental field. Herbicide efficacy was determined at 14, 28 and 75 days after treatment (DAT) by visual scoring against a range from 0 (no efficacy) to 100 (complete control). *Echinochloa* spp. density was assessed immediately before treatment and at 75 DAT.

The overall efficacy of penoxsulam-containing treatments was generally less than that obtained with cyhalofop-butyl applied alone or in combination with penoxsulam in this study. Profoxydim applied alone generally performed better than penoxsulam and cyhalofop-butyl applied alone, while no significant differences were found when the latters were applied in combination or as sequential application. Yet, in the third year of trial 1 the lowest efficacy was observed with profoxydim. In general, efficacy of all the treatments tended to decline over the years of the study. The average efficacy across all herbicide treatments recorded in 2012 at 75 DAT ranked in the following order, control in trial 3 (48%) was greater than trial 2 (28%), which was greater than trial 1 (8%). Cyhalofop-butyl efficacy was similar in the first and second years of application, while a substantial reduction in efficacy was recorded the third year. The efficacy of penoxsulam treatments was lower than those with cyhalofop-butyl in the first year and further reductions were observed in subsequent years.

In the non-treated plots, the density of *Echinochloa* spp. seedlings achieved before herbicide application dramatically increased after only one year without weed control, while an additional year without treatments resulted in only a small seedling density increase (19.5, 145.5 and 158.5 plants m<sup>-2</sup> at first, second and third year, respectively).

As a general conclusion, the repeated use of the same herbicide or mode of action for the control of *Echinochloa* spp. in the same plots across years resulted in reduced herbicide efficacy. Combination or sequential application of penoxsulam and cyhalofop-butyl provided more sustained high level of control than penoxsulam applied alone. The onset of apparent lack of efficacy reinforces the importance of rotating herbicide modes of action to sustain an adequate level of weed control across years or cropping cycles.

#### SENSITIVITY OF DIFFERENT ZEA MAYS SH2 GENOTYPES TO FORAMSULFURON

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The aim of this research was to study the mechanism involved in sensitivity of some maize genotypes to the herbicide foramsulfuron, and to examine its heredity. Foramsulfuron is a sulfonylurea herbicide for post emergence use in maize to control grasses and some broadleaf weeds.

Foramsulfuron is commonly formulated with the safener isoxadifen-ethyl (ratio 1:1). Four different *Sh2* maize genotypes - two homozygous inbred lines ('IBER001' and 'IBER002'), their hybrid ('ER00X') and a hybrid from a different genetic background ('PVS') - which showed significant differences in response to foramsulfuron + isoxadifen-ethyl, were tested in this research. 'IBER001' and 'PVS' were extremely sensitive to foramsulfuron + isoxadifen post application, with ED50 values of 3.6 and 43.6 g ha<sup>-1</sup>, respectively, while 'IBER002' and 'ER00X' were found to be resistant with ED50 values of 794 and 421 g ha<sup>-1</sup> respectively.

Differences in response to several other commercial herbicide formulations, with or without a safener, were examined, including ALS inhibitor formulations 'foramsulfuron + isoxadifen-ethyl', rimsulfuron, 'iodosulfuron + mefenpyr', 'foramsulfuron+ cyprosulfamid + iodosulfuron + thiencarbazone-methyl' and HPPD inhibitor formulation 'tembotrione + isoxadifen-ethyl'. 'IBER001' is highly sensitive to sub doses of all herbicides examined, while 'IBER002' and the hybrid 'ER00X' were tolerant to the recommended doses. 'PVS' hybrid was sensitive to 'tembotrione + isoxadifen-ethyl' but tolerant to all other formulations.

*In vitro* ALS inhibition by foramsulfuron was tested and no significant differences were found between cultivars. In addition, no mutations were present in ALS DNA sequence in the Pro197 or Leu574 sites. Malathion - a known cytochrome P450 inhibitor- was used to examine possible P450 involvement in the response of the genotypes to foramsulfuron. Pre-treatment with malathion significantly increased phytotoxicity caused by foramsulfuron to the resistant inbred and hybrid, but had no negative effect when foramsulfuron + isoxadifen-ethyl was applied.

Heredity of sensitivity to foramsulfuron was examined in F2 populations of the two hybrids. While F2 of 'ER00X' is a segregating population, with 1:3 sensitive to resistant ratio, 'PVS' F2 population is not segregating and all plants are sensitive to foramsulfuron.

'ER00X' F2 population is polymorphic in a SSR marker, previously showed to be linked to *nfs1* allele conferring sensitivity to P450 metabolized herbicides. Thus, our hypothesized mechanism of heredity in *Sh2* cultivars is a single recessive allele, conferring sensitivity to foramsulfuron. Recessive allele is possibly a mutated P450 encoding gene or transcription factor, thus causing sensitivity to other P450 mediated herbicides

Key words: Corn, selectivity, metabolism, P450, crop safety

#### MAIZE TOLERANCE TO HERBICIDES – ROLE OF THE SECONDARY METABOLITES

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Secondary metabolites play significant role in plant tolerance towards various stresses, including herbicides. Those metabolites mostly express antioxidant activity and variations in their content could be affected by various biotic and abiotic factors. Herbicide application could lead to stress in sensitive plants and affect antioxidant content. Maize inbred lines are much more sensitive than hybrid maize, due to their homozygosity. In the case of permanent stress plant are not available to overcome it, what could lead to yield decrease or its absence. The aim of this study was to examine and define tolerance of maize inbred lines towards sulfonilurea and triketone herbicides, based on alternation in secondary metabolites (phenolics, protein thiolic groups, phytic and inorganic phosphorus).

The reaction of five maize inbred lines (L1-L5) to triketones (mesotrione and topramezone) and sulfonylureas (rimsulfuron and foramsulfuron) was observed during three year field experiment. Herbicides were applied in recommended and double dose. Immediately after herbicide application (48h) plant samples were taken to estimate changes in secondary metabolites content. Visual damages (EWRC values) of maize plants were recorded in period of 2-3 weeks after herbicide application. Grain yield was measured at the end of growing season. Obtained data (three years averages) were statistically processed by ANOVA. The differences among tested secondary metabolites were evaluated using Principle Component Analyses (PCA). Dependences between EWRC values and content of secondary metabolites were obtained by regression analyses.

All tested lines had stronger damages when sulfonylureas were applied, compared to triketons. Triketons did not affect grain yield, while sulfonilureas decreased yield in the most treatments, especially in double dose. According to regression analysis, significant and negative correlation between EWRC values and free thiolics was present in L1, L2, L3 and L5, with the highest value obtained in L1 ( $R^2$ =0.719), also between EWRC and inorganic P int L4 and L5. The significant and positive correlation was observed between EWRC values and phytic P in L1, L2 and L3, with the highest value obtained in L3 ( $R^2$ =0.429). PC Analysis emphasized that free thiolics and inorganic P mainly contributed to PC 1, so they were commonly and in the highest degree affected by the herbicide stress. Phytic P mainly and negative contributed to PC2.

It could be concluded that the content of free thiolics was decreased in the highest degree by herbicide stress (mainly in sensitive lines), while inorganic P was mostly decreased in more tolerant lines. Increase in stress intensity was followed by the parallel increase in phytic P, irrespective to genotype sensitivity.

#### EFFICACY AND PHYTOTOXICITY OF HERBICIDES APPLIED PRE AND POST EMERGENCE IN LUPIN CROPS

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Lupin crops are an asset for sustainable cropping in rotations with cereal and oil seed crops. The lupin industry has been in decline over the last decades as a result of falling gross margins associated with a low seed price, increasing input costs and problem with effective weed control. As a rotation crop, they allow more options for control of grass weeds but there is no registered herbicide for postemergence control of broadleaf weeds.

The aim of this research was to determine the influence of different herbicides applied pre and postemergence. Field trials were conducted using lupins: narrow leaf (cv. Zeus), yellow (cv. Mister) and white (cv. Butan) grown at the Brody Research and Education Station of Poznan University of Life Sciences, during the 2012 and 2013 growing seasons. The soil type was luvisoil with pH range from 5.8 to 6.1. Fertiliser and agronomic practices were applied according to State Soil Testing Laboratory recommendations. The trials were set up as complete, randomised block design with four replicates and individual plot size of 2 m x 10 m. Treatments were sprayed in 200-L/ha volume and applied with flat fan nozzles and spray pressure set at 310 kPa. Visual assessment and reduction in bioassay of tested plants was determined as herbicide efficacy.

Lupin plants showed various tolerances to herbicides. The phytotoxicity was observed on lupins after postemergence herbicide treatments. The best weed control efficacy was observed when herbicide contains phenmedipham, desmedipham, ethofumesate and lenacil and was applied postemergence. These results demonstrate that above mentioned herbicides can be an effective for postemergence control of weeds in lupins especially broadleaf weeds. Therefore, to ensure effective control of all weeds, herbicide tank-mix should be used in combination with herbicides that have more activity on grass weeds. Using a combination of postemergence herbicides overcomes many of potential problems and also provides the best strategy for avoiding herbicide resistance. Selection will mainly depend on weed spectrum, cost, and use of restrictions.

# WEED CONTROL WITH POST-EMERGENCE HERBICIDES IN YELLOW LUPIN (*LUPINUS LUTEUS* L.) AND NARROW-LEAFED LUPIN (*LUPINUS ANGUSTIFOLIUS* L.)

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Due to their ability to fix atmospheric nitrogen, their positive effects on soil health and their nutritional value as a feed crop lupin has long been recognised as a valuable crop. Weeds are a major constrain in lupin. Prior to the introduction of herbicides, weeds in lupin were controlled mechanically by harrowing; inter row hoeing or hand weeding. The introduction of herbicides has enabled a significant expansion of the area cultivated with lupin and has resulted in significant yield increases. In Poland most herbicides recommended for weed control in lupin were introduced in the 1980s and 1990s. In recent years, the number of recommended herbicides has significantly reduced partly due to a low interest among agrochemical companies for developing pesticides for minor crop.

The aim of the study was to evaluate the possibility of dicotyledonous weed control with herbicides applied immediately after sowing (BBCH 0) or post-emergence at the 4-6 leaf stage (BBCH 14-16) of narrow-leafed and yellow lupin. The herbicides were applied alone or in tankmixtures. For pre-emergence treatment only linuron (590 g a.i./ha) was used. For post-emergence application the following herbicides were applied: diflufenican (80 g a.i./ha), metamitron (2100 g a.i./ha), lenacil (240 g a.i./ha), phenmedipham + desmedipham (160 + 160 and 320+320 g a.i./ha), phenmedipham + desmedipham (160 + 160 + 1400 g a.i./ha) and phenmedipham + desmedipham + diflufenican (160 + 160 + 80 g a.i./ha). Field experiments were conducted in 2010-2014 in two varieties of narrow-leaved lupin and yellow lupin. The most abundant weed species in the experiments were fat-hen (*Chenopodium album*), cornflower (*Centaurea cyanus*), black bindweed (*Fallopia convolvulus*), field pansy (*Viola arvensis*), and volunteer oilseed rape.

Post-emergence herbicides based on metamitron, diflufenican, lenacil were selective in lupin. highest efficacy against F. convolvulus was obtained using diflufenican The or lenacil. The efficacies of the herbicide against C. album, V. arvensis and volunteer oilseed rape were comparable. The use of post-emergence mixture of phenmedipham + desmedipham at higher doses and tank-mixtures of phenmedipham + desmedipham + diflufenican caused phytotoxicity to the crop and had a negative impact on yield. Narrow-leafed lupin was more sensitive than yellow lupin. Herbicides efficacy was influenced by weather conditions. In dry years herbicides efficacy against Ch. album was low with the exception of tank-mixtures of phenmedipham + desmedipham. Under dry weather conditions yield losses were lowest when mixtures of phenmedipham + desmedipham were applied.

In summary, the study has shown that post-emergence herbicide applications can control broad-leaves weeds in lupin grown under Polish conditions.

#### EFFECT OF SEED SIZE AND HERBICIDE PLACEMENT ON DRY BEAN INJURY FROM FLUMIOXAZIN

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Dry beans (*Phaseolis vulgaris* L.) are often injured from preemergence application flumioxazin, a protoporphyrinogen oxidase inhibitor herbicide. Factors including flumioxazin placement and timing, soil moisture and temperature, and bean market class and seed size reportedly influence bean injury. Green house trials were conducted to determine the effect of seed size and flumioxazin placement depth on subsequent bean injury. The injury response of four market classes of dry beans (great northern, black, navy, and pinto) to flumioxazin applied preemergence at 54 g ha<sup>-1</sup> to a silt loam soil were tested. Three cultivars differing in seed size within great northern and pinto market classes were included.

Placement of flumioxazin at seed depth (4 cm) greatly increased bean injury for all market classes (66 to 83% injury) compared to applying flumioxazin to the soil surface after planting (13 to 55% injury). Within great northern market class, large-seeded cultivar 'GN10-7' and small-seeded cultivar 'GN10-9' were more susceptible to injury from both surface applied and seed depth placement of flumioxazin than 'Matterhorn' with intermediate seed size. Within the pinto market class, larger-seeded 'Stampede' and smaller-seeded 'Medicine Hat' were more tolerant to flumioxazin than intermediate-sized pinto cultivar, 'PT11-34'. The two smallest seeded cultivars, navy bean 'Rexeter' and black bean 'Zoro' were among the most sensitive cultivars to flumioxazin applied to the soil surface.

Results demonstrate that herbicide placement in relation to seeding depth plays a larger role than seed size in determining dry bean injury from flumioxazin. Seed size influence on bean sensitivity to flumioxazin was not consistent within market classes. Genetic diversity among cultivars within dry bean market classes appears to influence sensitivity to flumioxazin more than seed size.

#### WEEDS CONTROL IN HARICOT BEAN SOWINGS UNDER THE FOREST-STEPPE ZONE CONDITIONS OF UKRAINE

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Legumes play a key role in protein production. Recently the farmers show more interest in growing of haricot bean in the country. Haricot bean is a minor crops, today the question of vegetative period and thresholds of harmful weeds as well as developing effective systems for their control by herbicides have not yet been investigated in Ukraine.

Field trials were carried out in 2014. Pre-emergence herbicides were applied at the day of planting, post-emergence – at the stage of 1-3 true leaves in haricot bean. A Sprayer - PL 2 "System Agrotop" with a water volume of 250 L ha<sup>-1</sup> was used. Herbicides efficacy was assessed 30 day after treatment (DAT) and before crop harvesting by measuring of weeds biomass.

It has been established that haricot bean plants have low competitive activity against weeds. Pre-emergence herbicides propizochlor, 1800 a.i. g.ha<sup>-1</sup>, acetochlor 1800 a.i. g ha<sup>-1</sup> and dimetanamid-P 720 a.i. g ha<sup>-1</sup> were most selective. The Efficiency of the herbicides was 88-94 %. Among post-emergence herbicides highest level of weeds control was provided by the application of imazamox 40 a.i. g ha<sup>-1</sup> or tank mixture of bentazone 720 a.i. g ha<sup>-1</sup> and tapraloxidim 54 a.i. g.ha<sup>-1</sup>. Weeds infestation level has been reduced by 88-91 % under these conditions. The highest yield 2.19-2.24 tha<sup>-1</sup> was obtained with propizachlor, 1800 a.i. g.ha<sup>-1</sup>, tank mixture of bentazone 720 a.i. g ha<sup>-1</sup> with tapraloxidim 54 a.i. g.ha<sup>-1</sup> and dimetanamid-P 720 a.i. g ha<sup>-1</sup>.

Applying pre-emergence propizachlor, acetochlor or dimetanamid-P or post-emergence use imazamox or tank mixture of bentazone with tapraloxidim gave reasonable weed control.

#### CHEMICAL CONTROL OF AMBROSIA ARTEMISIIFOLIA L. IN PRODUCTION OF FOREST SEEDLINGS

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Considering that quality of seedlings is one of the basic prerequisites for a successful establishment of plantations, production of seedlings in nurseries should be given special attention. Weed management in forest nurseries is very important because it is the quality of planting material that is a prerequisite for successfully establishment of forest plantations. At the Centre of Forest Nursery Production (44<sup>0</sup>49'10, 70"N 18<sup>0</sup>57'06, 96"E) high presence of *Ambrosia artemisiifolia* L. seriously competed forest seedlings.

For that reason, during the 2013-2014, experiments were established in order to investigate the possibility of control *A. artemisiifolia* L. in production of two-year old seedlings of black locust, pedunculate oak, sessile oak and ash. The investigated herbicides imazamox and clopyralid which are effective for the control of *A. artemisiifolia* L. and other large number broadleaf weeds. The experiments were established using randomized block design with four replicates. The efficiency and phytotoxicity of investigated herbicides was done 2, 4 and 6 weeks after treatments.

The used herbicides imazamox and clopyralid were effective in the control of *A. artemisiifolia* L. The clopyralid achieved efficacy from 80.5% to 92.0% but higher efficacy was achieved by applying the herbicide imazamox (83.0% - 98.5%). The investigated herbicides had no phytotoxic effect on two-year old seedlings of pedunculate oak, sessile oak and ash. However, imazamox and clopyralid showed phytotoxic effect on seedlings black locust. The results showed that imazamox and clopyralid can be applied for the control of *A. artemisiifolia* L. and other broadleaf weeds in production of two-year old seedlings pedunculate oak, sessile oak and ash but not black locust.

#### SENSITIVITY OF NATURALIZED *DIGITARIA AEQUIGLUMIS* POPULATIONS TO 4-HYDROXYPHENYL PYRUVATE DIOXYGENASE- AND ACETOLACTATE SYNTHASE-INHIBITING HERBICIDES IN MAIZE

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The boom of maize (*Zea mays* L.) cultivation in Flanders (Belgium) that started ca. four decades ago created optimal conditions for the establishment of permanent populations of many panicoid weed grasses (e.g. *Panicum* spp., *Echinochloa* spp., *Setaria* spp., *Digitaria* spp.) in and around maize fields. Until recently *Digitaria aequiglumis* var. *aequiglumis* (Hack. et Arechav.) Parodi, an alien species native to South America, was completely overlooked in Belgium due to its close morphological resemblance to *Digitaria sanguinalis* (L.) Scop. and *Digitaria ischaemum* (Schreb.) Mühlenb. *Digitaria aequiglumis* by now is firmly established and still under-recorded. One of the possible reasons for its expansion in maize fields, besides e.g. the lack of crop rotation, might be a lower sensitivity to post-emergence herbicides acting against panicoid grasses, in particular 4-hydroxyphenyl pyruvate dioxygenase (HPPD) and acetolactate synthase (ALS) inhibiting herbicides.

**Dose-response pot experiments were conducted in the glasshouse** to evaluate the effectiveness of four foliar-applied HPPD-inhibiting herbicides (topramezone, mesotrione, tembotrione, sulcotrione) and two foliar-applied ALS-inhibiting herbicides (nicosulfuron, foramsulfuron) for controlling Belgian populations of *D. aequiglumis*. Local *D. sanguinalis* and *D. ischaemum* populations were used as reference populations. Herbicides were applied at the three-leaf stage (BBCH stage 13). Fresh biomass was harvested 28 d after treatment. In another dose-response pot experiment, the influence of growth stage at time of herbicide application on efficacy of topramezone and nicosulfuron for *Digitaria* control was evaluated.

In general, sensitivity to mesotrione, tembotrione, sulcotrione, nicosulfuron and foramsulfuron was significantly lower for *D. aequiglumis* populations than for *D. ischaemum* and *D. sanguinalis* populations. Contrary to other herbicides tested, topramezone adequately controlled all *D. aequiglumis* populations at doses well below maximum authorized field dose. All species tested showed a progressive decrease in sensitivity to topramezone and nicosulfuron with seedling age. Four-leaf stage plants of *D. aequiglumis* were tenfold less sensitive to topramezone and nicosulfuron than plants at the one-leaf stage. A satisfactory post-emergence control of *Digitaria species* in the field will require appropriate choice of herbicide and dose, as well as a more timely application.

#### IONIC LIQUIDS BASED ON AUXIN-LIKE HERBICIDES

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Auxin-like herbicides (e.g. 2,4-D, MCPA. MCPP) are still widely used in agriculture due to low price, good effectiveness against dicotyledonous weeds, high level of selectivity for grasses, and low risk of selection of resistant biotypes. However, the disadvantage of these herbicides is their high volatility. This problem can be solved by transforming the active substance in the form of an ionic liquid.

In our study we synthesized many herbicidal ionic liquids (HIL) containing anions 2,4-D or MCPA. Specific quaternary ammonium salts having surface active properties were the source of cations. They were the following compounds: benzalkonium chloride, didecyldimethylammonium chloride, alkyltrimethylammonium chloride or alkylbis (2-hydroxyethyl) methylammonium chloride.<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were obtained for confirmation of the structure of new prepared ionic liquids. We determined the basic physicochemical properties of the synthesized compounds, such as glass transition temperature, crystallization temperature, melting point on heating, decomposition temperatures. All compounds were practically non-volatile. Biological activity of obtained ionic liquids was tested under field conditions.

The results of thermogravimetric analysis confirmed the high thermal stability of all synthesized ionic liquids.

The field trials were conducted in spring barley. The tested ionic liquids were applied at the end of tillering (BBCH 30) at rates ranged from 400 to 600 g a.i. per 1 ha. The efficacy of tested herbicides was determined four weeks after treatment. The results showed that 2,4-D and MCPA as ionic liquids gave excellent control of common lambsquarters (*Chenopodium album* L.), field pennycress (*Thlaspi avense* L.) and shepherd's-purse (*Capsella bursa pastoris* L.), they are herbicidal ionic liquids. Standards herbicide containing 2,4-D as the sodium salt and MCPA as the sodium-potasium salts were less effective. Moreover, it is interesting that studied HILs have shown much faster effects on weeds than standard herbicides.

All herbicidal ionic liquids were safe to spring barley plants. Our research has shown that new forms of auxin-like herbicides allow the use of reduced doses of the active substance and can be the useful tool in weed management.

#### MINERALIZATION OF <sup>14</sup>C-HEXAZINONE IN COMMERCIAL HERBICIDE MIXTURE WITH DIURON AND SULFOMETURON-METHYL

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Herbicides are the most widely used pesticides in agriculture and therefore are often found in studies of quality of surface and groundwater. The commercial herbicide mixture of diuron + hexazinone + sulfometuron-methyl is recommended in preemergence weed control in sugarcane. The herbicide hexazinone, one of the components of this commercial herbicide mixture is highly soluble in water (33 g L<sup>-1</sup>) and therefore highly mobile in the environment. This molecule is moderately toxic, but the presence of hexazinone can affect non target species. Previous studies already evaluated the fate of this herbicide after application in agricultural areas, but mineralization was not evaluated when it is applied in combination with others herbicides. Thus, this work studied the mineralization of the herbicide hexazinone applied alone and in mixture with diuron and sulfometuron-methyl in soils with clay and sand texture.

The study was conducted in the Laboratory of Ecotoxicology according to the guidelines of the "Organisation For Economic Co-operation and Development (OECD). The soils were collected from the upper 10 cm layer and then sieved though a 2 mm mesh and then, equivalent to 50 g airdry weight, was weighed into the 250-mL Bartha flask. Ten days before the application, soil moisture content was adjusted to 75% of the soil water-holding capacity and the flasks have been kept in room climatized to  $20 \pm 2^{\circ}$ C where the study was conducted. When applied alone non-radiolabeled solution was prepared according to the recommendation application dose of 391 g a.i. ha<sup>-1</sup> of hexazinone. When applied in mixture, non-radiolabeled solution was prepared plus an equivalent dose of 1391 g a.i.ha<sup>-1</sup> of diuron and 33 g a.i.ha<sup>-1</sup> of sulfometuron-methyl. To these solutions the <sup>14</sup>C-hexazinone was added and then the solutions were applied on top of soil, subsequently, the soil was homogenized. Mineralization of the herbicide was evaluated at 0, 7, 14, 21, 28, 35, 42, 63, 70 days after application. The <sup>14</sup>CO<sub>2</sub> released by <sup>14</sup>C-hexazinone was trapped in 0.2 N NaOH solution, which was analyzed for radioactivity content by liquid scintillation.

Total mineralization, at 70 days after the application, showed differences (p<0,05) between the applications of the herbicide alone (18%) and mixed with diuron and sulfometuron-methyl (21%) in sandy soil. The total mineralization was greater when the herbicide was applied to the soil with higher clay and organic matter, but with no differences (p>0.05) between treatments where the herbicide was applied alone (32%) or mixed (33%).

**SESSION IIB** 

WEED BIOLOGY

#### DIFFERENTIAL GERMINATION IN SEEDS PRODUCED IN APICAL AND BASAL FRUITS OF TWO THLASPI ARVENSE POPULATIONS

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*Thlaspi arvense* is a weed that is becoming rare in Spain, while it has also gained interest as a potential oilseed crop. The germination characteristics of seeds may be used to help select for crop improvement in several ways, one of them being improved crop establishment. The position of the seed in the mother plant can affect the dormancy level and, hence, its germination. In this work, the germination percentages and rates of seeds produced in the apical and basal fruits of the infructescences were studied on two *T. arvense* accessions, one from Spain and the other from USA, grown in the same field in Lleida (Spain).

Seeds of *T. arvense* were harvested selectively from mature apical and basal silicles of infructescences. The seven upper-most fruits on the main stem raceme of each plant were chosen as the "apical fruits", while the seven lower-most fruits on the main stem were chosen as the "basal fruits." These seeds were then used in germination experiments. Twenty-five seeds each of apical and basal seeds were sown in agar medium in each Petri dish, with four replications. Petri dishes were placed in complete darkness in growing chambers at 5, 8, 11, 14, 17 and 20° C constant temperatures. Germination was followed daily for 21 days. The experiment was repeated twice, from September to December 2013. Estimation of the T<sub>50</sub> for each temperature and its inverse  $(1/T_{50})$  allowed us to estimate the base temperature (T<sub>b</sub>) for each type of seed and population by linear regression.

Total percentage of germination was significantly higher in apical than in basal seeds, mainly at low temperatures (5 and 8°C) and in the Spanish accession. It was also significantly higher at 5 and 8°C than at temperatures higher than 11°C and in the USA accession than in the Spanish one. Generally, germination rates were faster in the apical seeds than in the basal ones, which indicate lower dormancy for seeds that have developed for a shorter time on the mother plant. Germination rates were also faster (despite lower percentages) at higher temperatures than at lower ones. Estimated  $T_b$  were 3°C for both apical and basal seeds in the Spanish accession.

These results indicate that dormancy levels clearly differ between apical and basal seeds due to maternal influence. Those seeds that were produced first (basal ones) acquire a stronger inherit dormancy that persists longer than those seeds produced later (apical ones). This information could be of value in order to select seeds for improved germination and crop establishment. The differences between populations are also relevant, indicating that some accessions might be more amenable for cultivation.

#### ASSESSMENT OF PHYLOGENETIC SIGNAL IN THE GERMINATION ABILITY OF BROOMRAPE (PHELIPANCHE RAMOSA) ON BRASSICACEAE HOSTS

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Assessing risks from a pathogen or parasite requires describing its full host range, which means cost-consuming experiments when the considered pathogen or parasite behaves as a generalist. Moreover, host ranges are likely to change over time, as new pathovars can be introduced, accidentally or after geographical expansion, and may also evolve locally. It is also known that host ranges often show some phylogenetic signal, i.e. the tendency of phylogenetically related host species to present more similar infections rates than host species drawn at random from the phylogenetic tree. Considering the phylogenetic structure of host species may therefore allow predicting which species are likely to be susceptible to a particular pathogen or parasite.

In France, winter oilseed rape is a new preferred broomrape [*Phelipanche ramosa* (L.) Pomel.] host, with a massive extension of the parasitic plant since the beginning of the 1990s, associated with yield losses over 80%. Numerous Brassicaceae weed species are known to be both infected by *P. ramosa* and abundant within oilseed rape fields, as selective herbicides for oilseed rape have a low efficacy on these closely related species. We recently demonstrated that *P. ramosa* is able to rapidly complete its life cycle on seven Brassicaceae weeds. Consequently, more attention should be paid to the role of Brassicaceae weeds that grow besides or after the crops as alternative hosts and to their importance for the demography of *P. ramosa*. The aim of our study was to characterize the germination of *P. ramosa* on a set of 14 Brassicaceae weeds and assess the presence of phylogenetic signal. The analysis of microsatellite markers we recently developed has confirmed the existence of at least three genetically distinct pathovars within *P. ramosa*, referred to as "Hemp", "Tobacco" and 'Oilseed-rape", according to the crop from which they were originally collected. Preconditioned seeds from those three pathovars were placed in contact with the roots of *in-vitro* cultivated individuals of Brassicaceae weeds and oilseed rape, and germinated seeds of *P. ramosa* were recorded after 3 weeks.

Based on a generalized linear model, germination counts were shown to vary significantly with the pathovar, the host species and their interaction. On average, germination percentages were higher for the pathovar "Tobacco" (34 % over all host species) as compared to "Oilseed rape" (18 %) and "Hemp" (15 %). Among host species, the most susceptible was *Cardamine hirsuta* (45 % germination for all 3 pathovars), while the least susceptible were *Raphanus raphanistrum*, *Rapistrum rugosum* and *Sysimbrium officinale* (< 10 % germination rate). A phylogenetic tree for the 14 Brassicaceae species, completed with *Brassica napus*, was constructed based on DNA sequences for the *rbcL* and *matK* genes. Phylogenetic signal, as estimated using Bloomberg's K was only significant for the pathovar "Oilseed-rape", which was also the one showing the highest variation in germination rate among host species. Results are discussed in relation with the recent origin of this pathovar.

#### EFFECT OF BURIAL DEPTH AND ENVIRONMENTAL FACTORS ON SEASONAL GERMINATION OF BEARDED SPRANGLETOP (*LEPTOCHLOA FUSCA*)

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Leptochloa fusca (bearded sprangletop) was recently introduced to Turkey, where it has apparently adapted to rice cultivation and become an important weed in many regions of the country. Experiments were conducted to determine the effects of light, temperature, osmotic stress, salt stress, pH, and flooding on germination and emergence of *L. fusca*.

Mature seeds of *L. fusca* were collected from rice fields in August, 2008. Approximately 1250 seeds were wrapped in plastic fabric and buried at 2 or 10 cm in pots under flooded and non-flooded conditions. Seed germination in light retrieved from flooded and non-flooded treatments began in spring, peaked in summer, and decreased in the fall. This pattern was repeated the following year after exposing seeds to natural seasonal temperature changes. Increased water stress or NaCl concentration resulted in decreased cumulative seed germination.

No seeds germinated when the NaCl concentration exceeded 400 mM. Seedling emergence decreased with increasing burial depth. Seedlings began to emerge at the end of April, continued until early July, and peaked in the first week of June under flooded conditions. Seedlings of non-flooded *L. fusca* emerged later than seeds under flooded conditions and overall emergence was low. Depth of burial and water stress appear to be the factors most limiting for seed germination. In our studies, germination was stimulated by light, suggesting that the seeds are positively photoblastic. Reduced soil tillage may reduce seed germination of *L. fusca* and may be incorporated into rice weed management programs.

#### STUDY OF GERMINATION PARAMETERS OF SUMMER ANNUAL WEEDS: TRANSFERABILITY OF ALERTINF MODEL TO CROATIA

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AlertInf is a prediction model for the emergence of weeds recently developed for five important summer annual weed species in maize and soybean fields in the Veneto region (Italy). The model is based on the hydrothermal time concept, which assumes that temperature and water potential have a primary influence on seed emergence. Thus, the application of this concept is possible when the specific biological thresholds are known. Consequently, the aim of this study was to provide a data set of base temperature and water potential for the summer annual weeds *Abutilon theophrasti, Amaranthus retroflexus, Chenopodium album* and *Echinochloa crus-galli* collected in continental Croatia. The comparison of the base parameters for Croatian and Italian ecotypes (estimated in a previous study) are used to evaluate the transferability of the AlertInf model to Croatia.

Three replicates of 100 seeds for each of the four species of Croatian ecotypes were incubated at a set of constant temperatures (8, 11, 14, 18, 21, 24, and 27 °C) and photoperiods of 12:12 h (light:dark) in petri dishes with deionized water to estimate base temperatures, and at 22 °C with different polyethylene glycol solutions (0; -0.05; -0.1; -0.25; -0.37; -0.5; -0.8; -1.0 MPa) to estimate base water potentials. Germination was recorded twice daily for temperatures of 21°C and higher, and water potentials above -0,37 MPa, or daily for lower temperatures and water potentials, until no further germination occurred for 10 days. The germination time course was analyzed using a logistic function and the reciprocal time of 50% of germination temperature or water potential. The base parameters were estimated as the intercept of the regression line with the temperature or the water potential axis.

The thresholds did not statistically differ between the Croatian and Italian ecotypes of *Abutilon theophrasti, Chenopodium album* and *Amaranthus retroflexus*. In contrast, *Echinochloa crus-galli* showed a lower base temperature in Croatia, confirming previous research that this species tends to develop ecotypes with a thermal response related to the climate of origin, i.e. in the cooler climate the base temperature is lower.

These findings are important as a first step for the transferability of the model from Italy to Croatia. The second fundamental step is to collect field emergence data and to estimate the specific parameters of the curve that describes the emergence dynamics of these summer weeds in maize and soybean crops in Croatia.

#### A SIMULATION MODEL TO PREDICT DORMANCY CHANGES IN UNDERGROUND BUDS OF SONCHUS ARVENSIS

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Sonchus arvensis is a problematic perennial weed in many crops in temperate area of the world. Information on its emergence timing is important to optimize weed management systems. Dormancy in underground buds is a characteristic that controls emergence timing of species in the field. A simulation model was developed to study dormancy development in underground buds of *S. arvensis*. The dormancy was hypothesized to be induced as a function of photoperiod and temperature, and released as a function of temperature. The model was parameterized and calibrated against observations of changes in dormancy in buds of plants growing in pots outdoors and exposed to natural conditions in Uppsala, Sweden.

The model calibration yielded a good prediction (R2=0.99; n=14) of changes in general pattern of dormancy in underground buds of the species. Dormancy induction started when the photoperiod or temperature was reduced below certain thresholds i.e. 16.5 hours day length for photoperiod and 16.8 °C for the temperature below which the accumulation of temperature for induction begins and reached its minimum threshold (0.4 GDD). The rate of dormancy induction increased proportionally with decreasing photoperiod and increasing accumulated temperature sum. The process continued progressively until the maximum dormancy induction was achieved. The maximum rates of dormancy induction due to the effect of temperature and photoperiod were estimated to be 0.2 and 0.7 per day, respectively. It is, therefore, concluded that the photoperiod plays by far a greater role than temperature for dormancy induction.

When the temperature decreased below a certain threshold (4.7 °C), the cold temperature was accumulated for dormancy release. When the accumulated cold temperature sum reached a certain threshold (-14 GDD), the dormancy started to release. Rate of dormancy release increased proportionally with the accumulated cold temperature sum until a threshold (-686.7 GDD) at which it reached its maximum rate and dormancy was fully released. The maximum rate of dormancy release due to the effect of temperature was estimated to be 0.3 per day.

The model was also validated against observations of bud dormancy in *S. arvensis* from a similar experiment. The model resulted in a good prediction of changes in a general dormancy pattern (R2=0.88; n=10). Therefore, the model can be used to predict and explain the seasonal dormancy pattern in underground buds of the species.

Dormancy as a survival mechanism enables the species to adapt the emergence period to an appropriate time for establishment and reproduction. Therefore, predicting the dormancy pattern is an important step to optimize the timing of weed control measures. It is concluded that the present model can explain the pattern of dormancy induction and release in underground buds and can further be used to improve the control of *S. arvensis* in agriculture.

#### EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF AMBROSIA CONFERTIFLORA

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Ambrosia confertiflora originating from southern US and Mexico, is a very aggressive and competitive perennial weed. The weed forms large stands reaching heights above 3 m with a very dense subterranean vegetative propagation system. In the last three decades Ambrosia confertiflora has invaded large areas in Israel, moving along river banks and roadsides. The highest concentration is in central Israel near the city of Nablus and along the Alexander river basin and the Heffer Valley.

In this study, we examined the development of plants grown under different temperature regimes in four growth Chambers: 28/34°C, 22/28°C 16/22°C and 10/16°C n/d. Plants and seeds were grown in each room and shoot elongation, biomass production, numbers of sprouts and seed germination were measured every week. Average results, standard error and regression were calculated for every experiment.

Results indicate that shoot elongation is inhibited by low (10/16°C and 16/22°C, n/d) temperatures and the plants remain in the rosette form. *A. confertiflora* reproduces from seeds and rhizomes. Seed germination occurs from the soil surface mostly at moderate temperatures (fall and spring). The numbers of sprouts with and without apex cutting (mowing) are not significantly different, indicating no apex dominance. The sprout number, however, is higher at cold conditions (10/16°C n/d). Biomass production varies at different temperatures, and is higher under warm conditions (22/28 and 28/34°C, n/d). *Ambrosia* species are wind pollinated, producing large quantities of highly allergenic pollen. *A. confertiflora*'s flowering season is between August and December. Pollen dispersal occurs mostly in the morning. We monitored the pollen quantities in the air in several locations in central Israel and found varying amounts of pollen in the air. Allergy skin tests were performed in two hospitals in Israel, using pollen extracts prepared from pollen collected in the field. Results from 162 volunteers exposed to different *Ambrosia* species show that 21% of the patients reacted at least to one of the species and that *A. confertiflora* is much more allergenic (17%) than *A. artemisiifolia* (5%). Our studies indicate that *A. confertiflora* poses a real threat to both annual and perennial crops as well as the health of the local population.

#### NORTHERN ADAPTATION OF SHORT-DAY WEEDS

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Climate change, characterized by higher temperatures and prolonged vegetation period, would increase the risk of a southerly weedy species establishing viable populations further north. However, if the species is a short-day plant, with a strong requirement for long nights for the induction of flowering, it might fail to produce viable seeds within the Scandinavian vegetation period. Thus, the invasiveness of such species in this specific region is dependent of their adaptation to northern photoperiod conditions. Genetic diversity in traits related to flowering time may, however, enhance adaptation.

Our main hypothesis is that the genetic diversity among and within populations regarding the requirement of a specific photoperiod for induction of flowering enables an adaptation of short-day plants to northern latitudes. To test it, we conducted two parallel common garden experiments with three weed species, which have been characterized as short-day species in at least some parts of the world: one already established in Swedish fields and a source of agricultural yield losses (*Chenopodium album* L.), one established in Sweden but, with few exceptions, only in waste lands and/or roadsides (*Amaranthus retroflexus* L.) and one species not yet established (*Ambrosia artemisiifolia* L.). The common garden experiments included 12-14 populations from European and North American countries for each weed species. The seed-populations were grown under similar soil and nutrient conditions in common gardens at high latitudes in Uppsala, Sweden (all three species; N 59° 48′ 55″, E 17 ° 38′ 47″) and at low latitudes in Osijek, Croatia (*A. artemisiifolia*; N 45° 31′ 16″, E 18° 40′ 54″) and in Dijon, France (*C. album* and *A. retroflexus*; N 47° 19′ 04″, E 05° 04′ 24″), as control.

In addition to the documentation of reproductive traits (time to bud, time to first male and female flower, time to present 50% of opened flowers, time to produce first mature seeds) in all species, DNA fingerprinting using microsatellites was conducted on all populations of *A. artemisiifolia* to measure their genetic diversity.

Preliminary results revealed that certain populations of *Ambrosia* are able to seed set under Scandinavian conditions and are, already now, pre-adapted to long-day photoperiodic conditions. Common general patterns of phenological variations in *A. artemisiifolia* and *C. album* showed a clear latitudinal gradient. For *A. artemisiifolia* this indicates a potential for fast adaptation and threat of a near future establishment.

#### INFLUENCE OF ENVIRONMENTAL FACTORS ON THE GERMINATION OF THREE BRAZILIAN RUBIACEAE WEEDS TOLERANT TO GLYPHOSATE

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The infestation of herbicide-tolerant species has increased greatly in recent years. From the North to the South of Brazil, it is becoming a major problem for farmers. *Borreria latifolia* and *Richardia brasiliensis* are important Rubiaceae weed species widely distributed in southern Brazil and are characterized by annual life cycles and reproduction by seed. *Galianthe chodatiana* is a Rubiaceae perennial species. It is not endemic in southern Brazil, and propagates by seeds and vegetative parts. These three weeds are tolerant to glyphosate and impact negatively the production of various crop species. Knowledge of factors that influence the processes of germination and dormancy and their interactions enable the development of predictive models of emergence and management practices that can be utilized to reduce infestations. This work was carried out to determine the effect of concentrations of aluminum, salinity levels and different pH's over germination and mean germination time (MGT) of these weed species.

Three studies (one for each species) were performed twice in the Plant Sciences Laboratory of the Federal Technological University of Paraná State, located in Pato Branco city, Brazil. Seeds of the three species were collected in soyabean crops of Paraná and Santa Catarina states, Brazil. The experimental units consisted of petri dishes with twenty seeds positioned on two filter-paper sheets and were located in growth chamber at  $25^{\circ}$ C and a photoperiod of 12 h. The experimental design was completely randomized with five replications, in a 3X5 factorial scheme (three species X five levels of factors). The aluminum effects were investigated using aqueous solutions with 0.0; 0.5; 1.0; 1.5 e 2.0 mEq Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>.18H<sub>2</sub>O /100 ml. Salinity effects were tested using aqueous solutions with 0; 25; 75; 150 and 300 mM NaCl /100 ml. Sodium hydroxide and hydrochloric acid were added to distilled water to obtain pH's 3.0; 5.0; 7.0; 9.0 and 11.0. The germination was assessed daily for 21 days. Thereafter the percentage of germination (PG) and MGTs were calculated.

All three species demonstrated a linear decrease of PG with increasing aluminum concentration. However, *R. brasiliensis* was more sensible than the other species. In relation to salinity, the PG of all species fitted a sigmoid model, with complete inhibition of germination at 300 mM NaCl/100 mL. *B. latifolia* was more sensitive with an approximately 50% decrease in PG at 75 mM NaCl and a 95% decrease in PG at 150 mM. As far as pH is concerned, the PG of all species fitted a polynomial quadratic model. The peak of *B. latifolia* PG ocurred at pH 3.0 while that of *R. brasiliensis* and *G. chodatiana* occurred at pH 7.0. The MGT showed a similar behavior compared to PG for all tested species and factors. The germination response of these Rubiaceae species to environmental factors can guide management programs aiming to reduce their infestations.

Keywords: Borreria latifolia, Richardia brasiliensis, Galianthe chodatiana, pH, salinity, aluminum.

#### EFFECT OF DIFFERENT TEMPERATURES ON THE GERMINATION OF EUPHORBIA MACULATA L., A NOVEL INVASIVE WEED IN THE NORTH OF IRAN

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Spotted spurge (*Euphorbia maculata* L.) is an annual plant in the family *Euphorbiaceae*, native to North America and recently it has become an invasive weed to soybean fields in the North-East of Iran. An experiment was conducted in 2014 in a germinator to evaluate the response and rate of seed germination of *E. maculata* exposed to seven temperatures: 0, 2, 4, 6, 8, 10, and 12 °C. 25 seeds of *E. maculata* were inserted in 9-cm Petri dishes and distrilled water used to keep seeds in suitable moisture to germination. Germinated seeds were counted daily and then removed from its Petri dish and data were recorded for 14 days. Six models including dent-like, segmented, beta, curvilinear, quadratic and third-degree functions were used to fit the regression.

Results showed that the percentage of seed germination in relation to temperature was best described by a beta function for *E. maculata*. Cardinal temperature estimation using the best models indicated that for *E. maculata*, the base temperature was 4.8 °C and the optimum and ceiling temperatures were 8.6 °C. The results indicated that the selected models, their parameters and cardinal temperatures could help in predicting the germination time of this weed under field conditions and choosing the optimum planting date of crops, because the main reason of its dispersion is the adoption to environmental conditions. Although many other factors may influence the germination and emergence in the field.

#### HOW SOIL DISTURBANCE AFFECTS THE EMERGENCE OF 30 RARE ARABLE PLANTS IN SPAIN

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Since they are the target of control measures, rare arable weeds suffer particularly from modern land use. In consequence, rare arable weeds belong to the most threatened plant groups in Europe. The regression of this segetal flora is due to changes in herbicide efficiency, nitrogen fertilization, seed sieving, soil cultivation and crop rotation among other factors. To preserve them, it is necessary to know their biology and response to the different management techniques used, like the type of soil tillage. This information is necessary at species level for conservation purposes. The objective of this research is to study the effect of soil disturbance on the emergence of 30 rare arable plants species at two burial conditions.

Seeds from 30 rare arable plant species were sown in late August during two consecutive seasons (2012/13 and 2013/14) at two depth conditions: constant 1 cm depth and homogeneously distributed from 2 to 10 cm depth. The experiment had a complete randomized block design with four repetitions per depth. Elementary plots were  $0.5 \times 0.5 \text{ m}^2$ . For each species 100 to 1000 seeds/plot were seeded. During both seasons, a tillage operation was simulated in early November in the plots with seeds sown from 2 to 10 cm depth. The total emergence per season was estimated through destructive counts. These trials were monitored a second season, disturbing the soil again in early November to simulate tillage. The effect of soil disturbance was analysed using R with Generalized Linear Models using a quasi-binomial distribution.

Species could be classified based on the results for different soil disturbances. In the first year of monitoring 8 species emerged more intensively with soil disturbance (i.e. *Hypecoum pendulum* or *Iberis amara*), and 13 without (i.e. *Roemeria hybrida* or *Camelina microcarpa*). When trials were monitored in the second year, only 2 species (*R. hybrida* and *C. microcarpa*) demonstrated significantly higher emergence rates without soil disturbance (14 vs. 7 and 6 vs. 4%, respectively), and 14 with soil disturbance (8 vs. 4%, on average). Seed traits like size could partly explain these results. Tillage had a species-specific effect on emergence and seed bank, and therefore, on plant biodiversity. This information, at species level, is necessary to understand how tillage affects these rare arable plants to preserve them in our agro-ecosystems. In coming seasons, more data will be gathered from these trials to understand how soil tillage affects the seed bank dynamics of these arable species.

#### EFFECT OF SOIL DEPTH ON THE GERMINATION OF SOME WEEDS IN THE SAHARAN AGROSYSTEM OF ALGERIA

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With the advancement of development in the region of southern Algeria, agricultural areas mainly concentrate on date palm, grain farming, market gardening and the cultivation of other crops. Investigations on the status of these production systems have shown the severity of pest problems in palm groves and pivots cereals. Weeds grow vigorously on farmland and are believed to be a major reason of species extinction and loss of biological diversity, just after the destruction of natural habitats.

Water pollution and concerns about the quality of agricultural products and increased herbicide resistance, led to the demand for alternative methods to the control of weeds with agrochemicals. These methods base on the knowledge of weed biology and factors promoting their distribution in Saharan agroecosystems.

The objective of this work is directed towards understanding the effect of soil depth on the emergence of weeds in the Saharan agro-system of Algeria. To carry out this work, we restricted our interest to some weeds of the Amaranthaceae family, namely *Amaranthus hybridus*, *Amaranthus albus* and *Beta vulgaris*.

Fifty seeds of these species were planted in pots at different soil depths: 0 cm, 5 cm, 10 cm, 15 cm and 20 cm. The germination rate was calculated at the end of the test for each species.

The results show that the rate of germination varies with depth and species. Germination rate decreases with increasing depth for all species. The effect of depth on the germination of *A. albus* is highly significant, the rate of germination decrease from 56, 66% at 0 cm to 26, 66% at 20 cm. While for *A. hybridus* and *B. vulgaris*, the effect of the depth on germination is very highly significant, the rate of germination decreases from 31, 33% at 0 cm to 4, 66% at 20 cm and from 84, 66% at 0 cm to 2, 66% at 20 cm respectively for *A. hybridus* and *B. vulgaris*. From the results it appears that, the germination rate is negatively correlated with depth.

Keywords: Agrosystem Saharan weeds, depth, germination, *Amaranthus albus*, *Amaranthus hybridus*, *Beta vulgaris*.
# SEED LONGEVITY OF CORTADERIA SELLOANA COMPARED WITH THE NATIVE SACCHARUM RAVENNAE IN THE VALENCIAN COMMUNITY POPULATIONS

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Biodiversity is mainly being threatened by two components of global change: mobility and landuse change. Disturbances are a key factor for the persistence and expansion of *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn. The high amount of seed rain from a few individuals benefits this species greatly when landscape changes, due to different disturbance patterns like land abandonment, habitat management or rubbish dumping. (Pausas *et al.*, 2006). Some *ex situ* techniques have been proposed to predict the persistence of weed seeds and enable land managers to make faster, better informed decisions for weed management programs (Panetta *et al.*, 2011).

This study focuses on the evaluation, through the seminal viability, the invasiveness capacity of *C. selloana* and the displacement of *Saccharum ravennae* (L.) P. Beauv. in protected marshes areas in the south of the Valencian Community (Spain). Following the protocol suggested by Probert *et al.* (2009), the relative seed longevity of the two species was measured by aging test (45 ° C and 60% RH). Seed viability was tested through germination percentages at 25°C and 12h photoperiod on agar (0.6%) during 15 days. The values obtained were adjusted by Avrami equation to generate curves of loss seed viability versus storage time and determine the P20, P50 and P80 parameters (Walters et al., 2004). A water sorption isotherm was calculated for both species at 20°C and 11, 15, 20, 30, 40, 50, 60, 70, 80, 85, 90 and 95% equilibrium moisture (Gold et al., 2007).

Results show that *C. selloana* had high water sorption capacity and lower seed longevity than *S. ravennae* (P50: 6.412 and 16.238, respectively). Those results support field observations and are positive for future eradication programs. However, additional studies, both *in situ* and *ex situ*, are needed to take into account all the factors involved in soil seed bank dynamics.

Keywords: Cortaderia selloana, Saccharum ravennae, ageing test, germination, invasiveness.

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# WEED SEEDS ABILITY TO EMERGE ON THE SOIL SURFACE

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Annual weeds have to produce seeds each year to maintain their populations. These seeds fall on the soil surface. Seeds exposed to light during their moistening (i.e. caused by rain) better germinate than seeds in the darkness (i.e. buried). However, rare studies quantified the unique and combined effects of light, moisture and burial depth on the germination process. We investigated, in a greenhouse experiment in 2014, the impact of seed moisture (*M*oistened vs. *Dried*), light during moistening (*Darkness vs. Light*) and burial depth (*Buried vs. Surface*) on germination of 12 annual weed species contrasted on their seed traits and germination periods (*Alopecurus myosuroides* Huds., *Amaranthus retroflexus* L., *Avena* fatua L., *Bromus sterilis* L., *Capsella bursapastoris* (L.) Medik., *Cyanus segetum* L., *Chenopodium album* L., *Geranium dissectum* L., *Poa annua* L., *Sonchus asper* (L.) Hill, *Veronica persica* Poir., *Vulpia myuros* (L.) C.C.GME L.). Six modalities were tested (with 5 replicates): *MDS* (moistened seeds in darkness and then placed on the soil surface), *MLS* (moistened in light and placed on the surface), *DLS* (dried in light and placed on the surface), *MDB* (dried in light and 0.5 cm-buried). *MLB* (moistened in light and 0.5 cm-buried), *DLB* (dried in light and 0.5 cm-buried). Datasets were analysed with generalized linear model (GLM, binomial distribution).

Results indicated that a strong variation of germination according to weed species. Globally, previously moist seeds (*M*) better germinated than previously dried (*D*) seeds (p-value <0.001) but light during the moistening did not increased the germination rate (MD=ML>SL) as expected. Contrary to our hypothesis, unburied seeds (*S*) better germinate than buried (*B*) seeds (p-value<0.001). The effect of each of the six modalities was investigated on the 12 species sub-datasets which showed strong differences in the germination patterns. We discuss here the effect of seed traits (size, soil-seed area contact, weight, ...) on germination patterns of unburied seeds.

**Keywords**: germination, emergence, burial depth, light, moisture, seed traits, no-till, conservation agriculture.

**Acknowledgements:** The experiment was financially supported by the ANR project COSAC and the Burgundy department (FABER program, CouvHerbi project S. Cordeau). Seeds were collected and freely provided by the 'weed seeds collection' group of the UMR1347 Agroécologie (INRA- AgroSup Dijon).

#### EFFECT OF IRRIGATION PERIOD AND SOIL DEPTH ON THE SEEDLING EMERGENCE OF CHENOPODIUM ALBUM

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Information on seedling emergence of *Chenopodium album* L. as one of the world's worst weeds is of vital value to optimize its management in cropping systems. Greenhouse and field experiments were conducted to study the effect of irrigation periods and soil burial depth on seedling emergence characteristics of *C. album*. Seed portions (100 seeds per pot and depth) of the species were buried in pots outdoors and in the greenhouse, at depths of 0, 2, 4, 6, 8, 10 and 12 cm in a strip plot design with four replicates in February 2013. Irrigations were included in 2-, 4- and 8-day periods. The number of emerged seedlings was counted and the emerged seedlings were removed regularly until the end of the experiment. Soil temperature was recorded at depths of 2, 6 and 10 cm during the field experiment. The temperature in the greenhouse was kept at  $17\pm2.6$  °C.

There was no difference in the total number of emerged seedlings for the various irrigation periods under field conditions. The maximum number of emerged seedlings (39-49% SEM) was observed when seeds were placed at the soil surface. The seedling emergence decreased with depth and reached its minimum (0.5-2%) at depths deeper than 6 cm. A similar pattern was observed in the greenhouse experiment. The number of emerged seedlings was slightly increased again at soil depths below 8 cm. There was also a significant difference among irrigation periods in the greenhouse experiment. Pots were sunk in soil in the field experiment while they were kept at ground surface in the greenhouse experiment. This might have facilitated drainage of water from pots in the greenhouse experiment and therefore highlighted the effect of irrigation periods.

In the field experiment, seedling emergence took place from late March until early June. Seedlings emerged over an extended period when seeds were placed at the soil surface or at shallower depths while the period of emergence was shortened with increasing depth. According to the recorded soil temperature, seedlings started to emerge when the daily mean soil temperature reached around 13 °C and tailed off when it reached more than 20 °C indicating an induction of secondary dormancy.

The cumulative seedling emergence was modeled using the Weibull function for seedlings emerged from depths of 2, 6 and 10 cm. The Weibull model allowed the prediction of the cumulative seedling emergence for all irrigation treatments ( $R^2$ =0.75-0.98). The emergence time was more delayed for seeds under every 2-day irrigation treatments than under 4- and 8-day treatments indicating that higher soil moisture content may delay seedling emergence of the species.

It is concluded that higher numbers of emerged seedlings observed in seeds placed at soil surface or shallower depths could be due to the stimulatory effect of light and higher temperature fluctuation on seed germination and consequently seedling emergence. The fluctuation in daily temperature was higher at shallower depth and decreased with depth. The seedling emergence window was also affected and reduced by soil depth.

Therefore, soil disturbance measures can be applied to manipulate the soil seed pool and consequently affect number and timing of seedling emergence and improve control of *C. album* under agricultural conditions.

# EFFECT OF IRRIGATION PERIOD AND SOIL DEPTH ON THE SEEDLING EMERGENCE OF AMARANTHUS RETROFLEXUS

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Amaranthus retroflexus is a C4 annual weed species that interferes with many crop production systems and causes yield reduction. Control of the species early in the season is of important value. To do so, information on its seedling emergence characteristics is necessary. Greenhouse and field experiments were conducted to study the effect of irrigation periods and soil burial depth on seedling emergence characteristics of *A. retroflexus*. Seed portions (100 seeds per pot and depth) of the species were buried in pots outdoors and in the greenhouse, at depths of 0, 2, 4, 6, 8, 10 and 12 cm in a strip plot design with four replicates in February 2013. The experiment included irrigation once in 2-, 4- and 8-days periods. The number of emerged seedlings was counted. The emerged seedlings were removed regularly until the end of the experiment. Soil temperature was recorded at depths of 2, 6 and 10 cm during the field experiment. The temperature in the greenhouse was kept at  $19.5\pm2.5$  °c.

There was no significant difference among irrigation treatments but the differences among soil depths were significantly different in the greenhouse experiment. In the greenhouse experiment, percentages of emerged seedling (32-40%) were higher when seeds were placed at the soil surface and at 2 and 4 cm depth at every 2- and 4-day irrigation treatments. However, it was slightly lower (28.5%) for seeds at soil surface and under every 8-days irrigation period. The degree of seedling emergence sharply decreased at 6 cm depth and reached its minimum at the rest of depths (4-1.5%).

In the field experiment, there were significant differences among depths and irrigation treatments as far as total percentages of emerged seedlings are concerned. The total number of emerged seedlings in every 8-day treatment was lower than in other irrigation treatments. Total percentages of emerged seedlings were higher for seeds placed at soil surface (31, 31.5 and 22.8% in 2-, 4- and 8-day treatment, respectively). Thereafter, the seedling emergence decreased with depth and reached its minimum at 6 and 8 cm depths, followed by a slight increase again at 10 and 12 cm depths.

Seedling emergence took place from March to late May in the field. According to the recorded temperature, seedlings started to emerge when the daily mean soil temperature reached around 10 °C and tailed off when it reached around 20 °C indicating an induction of secondary dormancy.

The cumulative seedling emergence was modeled using the Weibull function for seedling emergence from depths of 2, 6 and 10 cm. The Weibull model was suitable to predict the cumulative seedling emergence for all irrigation treatments ( $R^2$ =0.74-0.99). The emergence time was more delayed with an increase of depth and irrigation.

The numbers of emerged seedlings were substantially affected by burial depth. Delayed irrigation can also decrease the number of emerged seedlings. This information can potentially be used to apply soil disturbance measures in combination with irrigation periods to manage *A. retroflexus* in agriculture.

# SEEDLING EMERGENCE OF BRASSICA KABER AFFECTED BY SOIL DEPTH

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Information on seedling emergence from seeds of a weed species distributed in soil profiles is important to improve weed management strategies. A two-year field experiment was conducted to study the effect of soil burial depth on seedling emergence of *Brassica kaber*. Seed portions (100 seeds per pot and depth) of the species were buried in pots outdoors, at the depths of 0, 2, 4, 6, 8, and 10 cm, in a strip plot design with four replicates in January 2010 and 2011. Thereafter, the number of emerged seedlings were counted and removed regularly until the end of the experiment. The soil in the pots was kept moist and soil temperature was recorded at all depths during the experiment.

In 2010, the maximum seedling emergence  $(2.5\%\pm1\%$  SEM) was observed for seeds placed at the soil surface and emergence decreased with increasing soil depth  $(1\%\pm0.5\%$  at 2 cm depth). It reached  $0.5\%\pm0.4\%$  at a depth of 4 to 8 cm. Then the seedling emergence showed an increase again  $(1.8\%\pm0.8\%)$  at a depth of 10 cm. In 2011, the maximum seedling emergence  $(31.8\%\pm12\%)$  was observed for seeds placed at soil surface and decreased at the depths of 2  $(9\%\pm8\%)$ , 4  $(7.3\%\pm8\%)$ , and 6  $(5.8\%\pm5.5\%)$  cm followed by an increase at depths of 8  $(13\%\pm5\%)$  and 10  $(35.5\%\pm12\%)$  cm.

In 2010, first flushes of seedlings emerged in early February and the second flushes emerged in late April with fewer numbers for seeds placed at soil surface. Seedlings emerged sporadically at the rest of the depths and it took place from February to early May. In 2011, seedling emergence started with larger flushes in mid February, thereafter the emergence rates decreased until early April when the second flushes were observed for seeds placed at the soil surface. Seedling emergence tailed off in early May. A similar extended period of seedling emergence was observed for seeds at a depth of 2 cm but with fewer numbers of seedlings. The emergence period of main flushes was restricted mainly from late March to Early April at the rest of the depths. The results showed that seedlings started to emerge when the daily mean soil temperature exceeded 3°C and tailed off above 15°C indicating an induction of dormancy.

The result showed three depth responses regarding emergence including surface and shallower (larger numbers), medium (reduced numbers) and deeper soil depths (increased number). It is, therefore, concluded that higher numbers of emerged seedlings at the soil surface and at shallower depths could be due to a stimulatory effect of light or higher temperature fluctuation. The lower number of emerged seedlings observed in 2010 could be due to higher level of dormancy in freshly harvested seeds used in the first year of experiment. However, seeds used in the experiment in 2011, were dry stored at ca 5°C for a year that might have caused a reduction of their dormancy and consequently increased number of emerged seedlings.

# SEEDLING EMERGENCE UNDER NO-TILL CONDITIONS FROM INCREASING SEED BURIAL DEPTH

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The adoption of conservation agriculture can produce several economic and environmental benefits but implies modifications in terms of crop management. Weed control is a crucial aspect and, as mechanical control is limited or absent, dependence on herbicides increases especially during the transition from conventional to conservation tillage systems, because seeds of arable weed species remain viable in the soil while species adapted to no-till regimes simultaneously start to spread. The different arable weeds could present diverse adaptive ability to emerge under the conditions of no-till systems according to their specific biological and ecological characteristics. Information on the emergence dynamics of arable weeds during the transition period from inversion tillage to no-till conditions could contribute to the development of effective IWM strategies.

Two field experiments were conducted at Legnaro (Padova, Italy) to evaluate the ability of five troublesome arable weeds to emerge from increasing depths (1, 2, 5 and 10 cm) over a two year period of burial in simulated no-till conditions. The studied species were *Setaria viridis* (L.) Beauv. *Sorghum halepense* (L.) Pers., *Digitaria sanguinalis* (L.) Scop., *Amaranthus retroflexus* L. and *Abutilon theophrasti* Medik. Two hundreds seeds per replicate were sown on November 2006 and 2007 and there was no further soil disturbance throughout the duration of the experiments. Emerged seedlings were weekly counted and removed from March to the end of August for the two years following each sowing. Since no significant effect of the factor "experiment" was detected, data of the two experiments were pooled.

Seedlings mainly emerged during the first year after sowing and from depth of 1 and 2 cm. The inhibiting effect of increasing burial depth on seedling emergence varied among the different species with stronger effect on small-seeded species. The only exception was A. theophrasti, the largest-seeded species, which presented a notable depth-mediated emergence inhibition with total emergence of 10.9 % ± 1.20 SE from depth of 5 cm and no emergence from 10 cm. Sorghum halepense was the least affected species by burial depth, even emerging from a depth of 10 cm and having the highest total seedling emergence (42.8  $\% \pm 4.86$  SE). Amaranthus retroflexus was instead highly affected by burial depth, with no emergence from depths of 5 and 10 cm and the lowest total seedling emergence (10.0 %  $\pm$  2.71 SE). Differences in emergence percentage as affected by burial depth and no-till conditions were obtained among species, showing their diverse adaptive ability to these specific environmental conditions. The lack of stimuli (light, oxygen, temperature fluctuation) required by buried seed for dormancy break and germination has been reported as a possible cause of depth-mediated inhibition of seedling emergence under no-till conditions. Analyzing how no-till conditions influence affect dormancy cycle of buried weed seeds could facilitate the creation of effective IWM strategies for the transition period from conventional to conservation tillage systems.

# VARIABILITY OF GERMINATION RESPONSE OF EUROPEAN POPULATIONS OF ABUTILON THEOPHRASTI AS A FUNCTION OF SEED MATURING SEASON

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Inter-population variability regarding dormancy and germination has been considered as part of the adaptive strategy of weeds to spread across areas with different environmental conditions or with frequent anthropic disturbances. Intra-specific variability has been observed among weed populations within the same climatic region as a consequence of specific local environmental and agronomic conditions. Acquiring knowledge about intra-specific variability of the main weeds species could contribute to develop effective and transferable IWM strategies.

Seeds of three populations of *Abutilon theophrasti* Medik. were collected in summer 2008 at three locations: Legnaro (Padova, Italy), Tapada da Ajuda (Lisbon, Portugal) and Arganda del Rey (Madrid, Spain). The three seed batches were sown in autumn 2008 at the CSIC-experimental farm located in Arganda del Rey, so all emerged plants experienced the same maternal environment during their life cycle. Seeds were collected in 2009 in three subsequent moments, called Collection Times (CT 1, 2 and 3), i.e. 9<sup>th</sup> July, 27<sup>th</sup> August and 16<sup>th</sup> October, to compare germination response of seeds produced under different maturing season (i.e., environmental conditions). Nine seed batches were obtained. Mechanical scarification was performed on seeds to facilitate imbibition. Seeds were incubated at different constant temperatures (8, 12, 18 and 22 °C) with 12 h of light. Three 50-seed replicates were included for each treatment. Germinated seeds were counted and removed daily and at the end of the experiment viability of nongerminated seeds was checked with a seed-crushing test. The germination percentage of each replicate was calculated with the total number of viable seeds as a basis.

The germination percentage reached maximum values at the central incubation temperatures (12 and 18 °C) while it decreased at 8 or 22 °C. The mean total germination percentages of the three populations reached similar values close to 90 % if results of seed batches with different CT were averaged. Instead, significant differences were detected among the germination responses of seed batches derived from the same populations but collected at different moments. Seed batches CT3 of the Italian population and CT1 of the Spanish one presented lower total percentages of germination (78.1 %  $\pm$  2.34 SE and 81.5 %  $\pm$  4.55 SE respectively) than the other seed batches from the same populations. These differences increased at the lower and higher incubation temperature with germination percentages of around 70 % for both seed batches. The seed batches CT3 Italian and CT1 Spanish were therefore probably characterized by a higher level of dormancy than the other seed batches produced at different moments by the same populations or at the same moment by different populations. The highest and most constant germination response was obtained by CT2 for the Italian and Portuguese population and by CT3 for the Spanish one. Further studies are required to asses if the observed variability represents part of the adaptive response of the three populations to their specific local environmental conditions.

# EFFECT OF THE RIPENING SEASON ON THE GERMINATION RESPONSE OF DATURA STRAMONIUM POPULATIONS

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Variability regarding dormancy and germination has been observed among some European populations of *Datura stramonium* L. Gradual adaptive processes to local environmental and agronomic conditions are supposed to be related to this intra-specific variability. Moreover, differences could exist among seed batches produced by the same plants in different seasons according to the specific environmental conditions during their ripening. The interaction of these two phenomena probably determine the intra-specific variability of local *D. stramonium* populations; a specific study on this interaction could therefore contribute to expand the existing knowledge about this important weed.

Seed collections of three populations of *D. stramonium* took place in autumn 2008 from maize fields at Legnaro (Padova, Italy), Tapada da Ajuda (Lisbon, Portugal) and Arganda del Rey (Madrid, Spain). The three seed batches obtained were sown in autumn 2008 at the CSIC-experimental farm located in Arganda del Rey, so differences regarding maternal environment during life cycle of the following plants were minimized. The seeds were collected in 2009 at three subsequent moments, called Collection Times (CT 1, 2 and 3), i.e. 18<sup>th</sup> September, 16<sup>th</sup> October and 10<sup>th</sup> November, to compare germination response of seeds produced during the whole ripening period. Nine seed batches were obtained. Small portions of seed coat were mechanically removed by pricking before germination tests to facilitate seed imbibition. Seeds were incubated at different constant temperatures (8, 12, 18 and 22 °C) with a photoperiod of 12/12 h light/darkness and three 50-seed replicates for each treatment. Germinated seeds were counted and removed daily and at the end of the experiment. Non-germinated seeds were checked to assess viability with a seed-crushing test. The germination percentage of each replicate was calculated based on the total number of viable seeds.

The germination percentage reached maximum values for all seed batches at the highest incubation temperatures (18 and 22 °C) while values were significantly lower at 12 °C and no germination occurred at 8 °C. Mean total germination percentages varied from 59.2 %  $\pm$  6.70 SE for the Spanish population to 50.6 %  $\pm$  6.43 SE for the Italian one, but no statistical differences were detected. The Portuguese population instead presented the lowest germination percentage (20.9 %  $\pm$  3.77 SE) at 12 °C temperature at which the greatest differences in germination response among the three seed batches in terms of CT were detected. Seed batches CT1 of each population reached a higher germination percentage than the others, while CT3 reached the lowest. Seed batches CT3, which were produced at the end of the growing season, were probably characterized by a higher degree of dormancy, especially the CT3 Italian which resulted in a lower germination percentage also at 18 °C. Seeds produced during ripening season CT3 largely increased the variability observed regarding dormancy and germination even within the single populations. Since this situation was similar among the three populations, a certain role in adaptive process could therefore be supposed.

# EFFECT OF DROUGHT ON STOLON GERMINATION OF SPRANGLETOP (*LEPTOCHLOA FUSCA* L.) UNDER NATURAL CONDITIONS

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Sprangletop (*Leptochloa fusca* L.) is a perennial or biennial plant from the *Poaceae* family that has been problematic in sugarcane fields in the south of Iran. Understanding of biological and ecophysiological characteristics of this plant is essential to prevent its dispersion into other areas. The most critical period of the life cycle of plants, especially invasive plants, is germination. To get this information, an experiment was conducted in 2013 at the Sugarcane and Byproduct Research and Training Institute of Khuzestan, Iran. The experiment was conducted in a completely randomized design with three replications. The first factor was bulk density of stolons in 2 levels (2 groups sorted by bulk densities 0.06 and 0.08 g cm<sup>-1</sup>) and the second factor was time of placement in nature exposed to direct sunlight for 0 (control), 3, 6, 9, 12, 15, 24 and 32 hours. Germination percentages were monitored every 24 h for 7 days. The stolons were considered to have germinated after radicle emergence (2 mm length). Stolons that had already germinated were counted and discarded when germination number x100 / total number of germination. The rate of germination was number of germinated stolons in every day.

Results showed that bulk density of stolons did not influence the rate of germination, but influenced the percent of germination. Time of exposure to direct sunlight affected both rate and percent of germination. Discrimination between the two factors (interaction between bulk density and exposure) showed that the rate of germination was affected but the percentage of germination was not affected by this interaction. Stolons with more bulk densities have a higher percentage of germination and 0 (control) and 3 hours of exposure in nature had the highest percentage of germination. In both bulk densities, 0 (control) and 3 h exposure in nature showed the highest rate of germination. The least rate of germination for stolons with 0.06 and 0.08 g cm<sup>-1</sup> were seen 9 and 32 hours after placement in nature, respectively. Information about trends of changes in rate and percentage of germination (that were deceased by time of exposure to sunlight) and changes in climate, lead us to propose using tillage as a convenient weed control method. However, it is important to apply this method in a scientific way and cut the stolons in short pieces, because short stalks (stolons) have less moisture and will be controlled better by increasing time of exposure to direct sunlight.

#### ALLELOPATHY OF TREE OF HEAVEN (*AILANTHUS ALTISSIMA*), COMMON MILKWEED (*ASCLEPIAS SYRIACA*) AND GIANT HOGWEED (*HERACLEUM MANTEGAZZIANUM*) ON THE GERMINATION OF WINTER WHEAT

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The weed species Ailanthus altissima [Mill.] Swingle (tree of Heaven), Asclepias syriaca L. (common milkweed) and Heracleum mantegazzianum Somm. Et. Lev. (giant hogweed) have been spread in Hungary in recent years and can cause several harmful effects. A. altissima is a rapidly growing deciduous tree and produces allelochemicals in its roots that inhibit the growth and germination of many plants. A. syriaca is one of the most dangerous weed species in our country, often causing damages in arable fields. H. mantegazzianum can only be found sporadically in Hungary. This weed has allelopathic as well as strong human allergenic effects.

Allelopathic effects of these three species on winter wheat (Lupus) were studied in bioassays in 2013 and 2014. Water extracts were made from collected and frozen shoots of weeds in three concentrations: 2.5%, 5% and 7.5%. The extracts were filtered after 24 hours, and were used immediately. Fifty pieces of winter wheat seeds were placed in Petri dishes and treated with 15 ml solutions. In control dishes, 15 ml tap water was used. They were put into thermostat and held in dark. All treatments were made in four replications. The germination percentage was studied and the whole length of the primary roots and shoots was measured on the 8<sup>th</sup> day of the assay. The statistical analysis has been made using the SPSS computer program, two-factorial variance analysis.

Studying the effect of extracts on the germination percentage of wheat, we found that *A. altissima* decreased it in the largest amount, followed by *H. mantegazzianum*, while *A. syriaca* hardly influenced it. Solutions that were more concentrated evoked stronger inhibitory effect. Shoot lengths were significantly (LSD<sub>5%</sub>) decreased by the 7.5% concentration of extracts of all three weed species. *A. altissima* shoot solution showed the strongest inhibitory effect. The control plants had 121 mm long shoots while the treated ones had only 89 mm. Solutions that were more concentrated (5%), decreased the length also significantly. The second in effectiveness was *A. syrica*, followed by *H. mantegazzianum*. On the contrary, root development was stimulated by 2.5% solutions. It was outstanding in the case of *H. mantegazzianum*, with 27% longer roots. The next was *A. altissima*, and the last was *A. syriaca*. While the 5% solution of *A. syriaca* increased the root length, 7.5% concentration hindered the growth of roots significantly, with the strongest decrease obtained by *H. mantegazzianum* (from 131.43 mm to 128.07 mm).

Our results documented the allelopathic effect of the examined three weed species. Solutions that were more concentrated inhibited the germination and growth of winter wheat strikingly. As opposed to this, diluted extracts stimulated the root growth.

Present article was published in the frame of the project TÁMOP-4.2.2.A-11/1/KONV-2012-0064. The project is realized with the support of the European Union, with the co-funding of the European Social Fund."

### DIFFERENTIATION OF WILD OAT (AVENA SPP.) SPECIES IN TURKEY BY CLASSICAL AND MOLECULAR METHODS

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Wild oats (*Avena* spp.) are troublesome weeds in their native and nonnative range in wheat fields as well as in other cropping areas. In this study the economically important wild oat species in Turkey are identified which is important in order to develop control strategies. Despite the presence of eight different wild oat species documented in Turkey, *Avena fatua* L., *A. sterilis* and *A. ludoviciana* are seen as a problem in wheat fields. Herbicides against one or more of these species are registered. Two out of three species are considered as subspecies of *A. sterilis*: *A. sterilis* is a synonym of *Avena sterilis* L. subsp. *sterilis* L. and *A. ludoviciana* is a synonym of *Avena sterilis* L. subsp. *ludoviciana* (Durieu) Gillet et Magne. Also, *A. sterilis* and *A. ludoviciana* resistance data were evaluated separately.

In the first outcomes, morphological data and molecular data are parallel. Molecular methods were applied to differentiate 200 wild oats populations which were collected in wheat fields from all geographical regions in Turkey. These populations were identified with molecular and classical method. In molecular studies silica capture method and direct DNA extraction procedures were used to extract nucleic acids of *Avena* species. Specific primers were designed by us using the primer designing programs with the following parameters: Tm range 50 to 60°C, CG Range 50 to 60%, primer length 18 to 24 and Tm within 5°C. 3 primer pairs designed for each Avena species using the genbank of NCBI. PCR conditions were optimized according to primer properties.

Keywords: Avena, identification, PCR.

# NO EVOLUTIONARY SHIFT IN THE MATING SYSTEM OF THE INVASIVE WEED AMBROSIA ARTEMISIIFOLIA POPULATIONS IN FRANCE

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Autogamy allows the reproduction of isolated plants so that these plants can produce a new population. On the other hand, allogamy tends to increase the genetic variability in plant populations and by processes of admixture, generates recombinant genotypes which can be adapted to new environments.

Common ragweed (*Ambrosia artemisiifolia, Asteraceae*) is an annual herb mostly known in Europe as an invasive plant originating from North America that colonizes different types of habitats (railways, river sides, wastelands and farmlands) including cultivated fields.

It is commonly accepted that the mating system plays a key role in plant invasion processes. *Ambrosia artemisiifolia* has a strong self-incompatibility system and presents high outcrossing rates in its native area in North America and in a colonized range in China. Our purpose was to examine some mating system parameters in order to determine whether there has been an evolutionary shift towards selfing (or partial selfing) in *A. artemisiifolia* populations in France.

The mating system of eight *A. artemisiifolia* populations was investigated using newly developed nuclear microsatellites markers. The populations were sampled in an agricultural landscape situated southeast of Dijon (Burgundy, France). Most populations were located along cultivated field margins or within cultivated fields. The values of the multi-locus outcrossing rate (*tm*) and maternal inbreeding coefficient were not significantly different from 1 and 0 respectively. These results indicate that *A. artemisiifolia* has a completely outcrossing mating system in the studied area. Outcrossing rates between related individuals (*tm-ts*) were significantly different from 0 for several populations, indicating some degree of biparental inbreeding in these populations. The correlations of paternity were significantly greater than 0 suggesting moderate numbers of pollen donor parents. Genetic diversity was high in all populations studied. Genetic differentiation was low ( $F_{ST} = 0.058$ ) but significant and patterns of genetic structure were identified revealing distinct colonization events. The level of genetic admixture of plants was variable and high in several populations. We hypothesize that a higher degree of admixture was associated with a longer time since the populations were founded.

Our results demonstrate that, in the agricultural landscape we surveyed, there has been no evolutionary shift towards autogamy in the mating system of *A. artemisiifolia*. The successful spread of *A. artemisiifolia* at the landscape scale was facilitated by multiple introductions followed by genetic admixture. Nevertheless, the issue of isolated plants should be raised and the mating system of these plants will be investigated.

# **SESSION IIIA**

# HERBICIDE RESISTANCE

# MANAGING ALS-RESISTANT BROAD-LEAVED WEEDS: CURRENT RESEARCH AND FUTURE NEEDS

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World-wide, the acetolactate synthase (ALS-inhibiting) herbicide group has the greatest incidence of resistance and no new herbicide modes of action are on the horizon. Broad-leaved weed resistance is currently at a low and manageable level in the UK and predominately includes *Papaver rhoeas* (common poppy), *Stellaria media* (chickweed) and *Tripleurospermum inodorum* (scentless mayweed). However there is a heavy reliance on ALS-inhibiting herbicides due to limited choice from other modes of action and so the risk of broad-leaved weed resistance increasing is extremely high.

A research project is being delivered through a series of container and field-based experiments, currently in the third experimental year, to provide more detailed data and understanding focusing on *P. rhoeas*. Container-based trials include three *P. rhoeas* populations, two resistant populations (one collected from the field trial location) and one susceptible. For both the container and field experiments a range of pre- and post-emergence herbicides have been applied over a three year period to assess how rapidly resistance builds up with each herbicide programme. The herbicide treatments include (1) ALS-inhibitors alone, (2) non-ALS + ALS and (3) all non-ALS.

The aim of the research is to (1) To identify and quantify the risks of ALS resistance in broadleaved weeds; (2) To develop the optimum management practices to manage, reduce or eliminate developing resistance levels; (3) To raise awareness of the issue in the UK and provide information about the early warning signs and how to manage the situation.

Initial results show that ALS resistant *P. rhoeas* can be controlled by non-ALS inhibitor herbicides. Timing of herbicide application is critical for effective weed control as the *P. rhoeas* population was extremely high and growth stage was very variable in the field. The use of an ALS-inhibitor alone had no effect on the resistant *P. rhoeas* leading to a rapid build up of seed in the weed seedbank as seed production and longevity is so high for this species. Management challenges for resistant broad-leaved weeds are almost greater than those for grassweeds as cultural control methods are not always as effective, putting more pressure on the herbicides.

Wider communication of this research has resulted in networks and contacts across Europe, highlighting the interest and concern for broad-leaved weed resistance and for resistance testing programmes to not be restricted to individual countries. This paper will explore more detail of species tested, results and implications for improved prediction and management of broad-leaved weed resistance.

# IMPACT OF THE AMINO ACID SUBSTITUTION ASP376GLU ON THE EFFICACY OF ACETOHYDROXYACID SYNTHASE INHIBITING HERBICIDES IN *LOLIUM SP.* (L.)

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Lolium sp. (ryegrass) is one of the main monocotyledonous weeds found in small grain cereal cropping systems in France. The overreliance on AHAS-inhibiting herbicides in cereals for ryegrass control has resulted in the evolution and spread of resistance over the past 10 years. Cross- and multiple-resistance patterns, based on target-site (TSR) and non-target-site (NTSR) mechanisms. are complex and may affect sulfonylureas, imidazolinones, resistance triazolopyrimidines and sulfonyl-carbonyl-triazolinones performance. So far, only single nucleotide polymorphisms at position 197 and to a lesser extent at position 574 of the ryegrass AHAS-gene have been documented in France. Amongst the single nucleotide polymorphisms known to confer resistance against AHAS-inhibitors, the aspartate to glutamate substitution at position 376 was reported in dicotyledonous species since 2004. The Asp376Glu substitution was recently documented in Amaranthus hybridus, where it was found to confer resistance to imidazolinones sulfonylureas (imazethapyr), (chlorimuron and thifensulfuron) and triazolopvrimidines (cloransulam). Three ryegrass populations sampled in 2012 in France were found to bear the Asp376Glu mutation. This is the first known occurrence of Asp376Glu in ryegrass species. Therefore cross-resistance patterns against sulfonylureas, imidazolinones, triazolopyrimidines and sulfonylamino-carbonyl-triazolinones conferred by Asp376Glu in ryegrass are unknown.

Seed samples of the mentioned populations were cultivated under greenhouse conditions and treated with 100 g a.i. ha<sup>-1</sup> imazapyr for selection of resistant individuals. Survivors where genetically characterized by sequencing of the AHAS-gene between nucleotides 122 and 654. Plants bearing the Asp376Glu mutation were multiplied by repeated tiller propagation for herbicide efficacy trials and pleiotropy studies. All selected Asp376Glu genotypes were heterozygous and showed no other single nucleotide polymorphism on the AHAS- and ACCase-gene. Furthermore all selected genotypes were sensitive against ACCase inhibitors (aryloxyphenoxy-propionates, cyclohexanediones and phenylpyrazolines), and thus the probability for masked NTSR mechanisms biasing the resistance profiles of the selected genotypes can be excluded. A sensitive reference genotype was selected out of the same initial population as well as two resistant reference genotypes bearing the Trp574Leu hetero- and homozygous.

Plants bearing the Asp376Glu mutation were highly resistant against mesosulfuron, pyroxsulam and propoxycarbazone, whereas against imazamox only moderately increased tolerances for dosages below 140 g a.i. ha<sup>-1</sup> were observed. The Asp376Glu genotype showed significantly reduced growth height, while above ground biomass accumulation and the number of tiller per plant were not significantly different compared to the genetic wild type. Although the differences were not significant, it has to be emphasized that root biomass accumulation of the Asp376Glu genotype was reduced by more than 50% compared to the genetic wild type. This study highlights the role of the Asp376Glu mutation on the resistance profile against AHAS inhibiting herbicides in ryegrass. However, the observed pleiotropic effects of the Asp376Glu mutation are providing a first indication that plant fitness is hampered.

# MONITORING OF RESISTANCE DEVELOPMENT AGAINST ALS INHIBITING HERBICIDES IN DICOTYLEDONOUS WEEDS IN GERMANY

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The number of weed species resistant to herbicides belonging to the group of ALS inhibitors has been steadily and dramatically increaseing during recent decades. By now this increase appears to be slowed down but is still progressing. In Germany, mainly grass weeds such as *Alopecurus myosuroides* Huds. and *Apera spica-venti* (L.) P. Beauv. were shown to be resistant to herbicides containing ALS-inhibiting active ingredients. But recently, the first dicotyledonous weeds also gained attention for their potential inherent risk to develop resistance mechanisms against ALS-inhibiting herbicides. *Tripleurospermum perforatum* (Mérat) Lainz and *Stellaria media* L. (Vill.) s. str. were found to exhibit resistance to ALS inhibitors probably due to an altered target site. Apart from that ALS inhibitor resistant *Papaver rhoeas* L. and *Amaranthus retroflexus* L. biotypes were shown. In these species the resistance mechanism has not yet been classified. A monitoring project was therefore initiated in to further elucidate the degree and distribution of dicotyledonous weed species resistant to ALS-inhibiting herbicides in Germany. Field seed samples from *A. retroflexus, Centaurea cyanus* L., *Matricaria recutita* L., *S. media, P. rhoeas* and *T. perforatum* were collected.

Weed species collected for this monitoring were grown under controlled conditions in the greenhouse or in plant growth cabinets. Herbicides containing ALS-inhibiting active ingredients were chosen according to their efficacy on the target weed species. Herbicides were applied at maximum field rate at BBCH 10-14 of the plants. The efficacy of the herbicide treatment and plant survival were determined 21 days after herbicide treatment.

All biotypes of *A. retroflexus*, *S. media*. and *T. perforatum* were successfully controlled by the herbicide treatments. For *P. rhoeas* one population was found to exhibit reduced sensitivity to the tribenuron and florasulam while another population was found to be resistant to the both active ingredients. Resistance in the latter biotype was found to be due to a P197S substitution in the ALS gene. In addition, three *M. recutita* populations resistant to tribenuron were detected. Here, the mechanism of resistance is still to be elucidated and experiments are ongoing. Nevertheless, target-site resistance is suspected. Additionally, single plants from two other *M. recutita* populations also survived the treatment with the active ingredient florasulam. *C. cyanus* was successfully controlled by the applied herbicides but single survivors were found. The mechanisms for plant survival are still to be elucidated.

In summary, only few weed populations exhibiting resistance to ALS-inhibiting herbicides were identified in this monitoring. Summarising these results with data received from a resistance monitoring on *Matricaria* species in 2011, it is suggested that the level of herbicide resistance to ALS-inhibiting active ingredients is still moderate in dicotyledonous weed species in Germany.

# PATTERNS OF CROSS-RESISTANCE TO ACCASE-INHIBITOR HERBICIDES IN WINTER WILD OAT (AVENA LUDOVICIANA) POPULATIONS

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The level of resistance and patterns of cross-resistance to clodinafop-propargyl, sethoxydim and pinoxaden were examined in 12 putative resistant and one susceptible population of winter wild oat (*Avena ludoviciana* Durieu.) collected from Iran. The responses of biomass, plant survival, and coleoptiles to the increasing dosages of the three herbicides were determined in whole-plant and seed bioassays, respectively.

In whole-plant bioassay, all 8 putative resistant populations were indeed confirmed resistant to clodinafop-propargyl with resistance ratios ranging 3.1 to >34.1 or 2.57 to >50.6 for biomass and survival data, respectively. Most clodinafop-resistant populations exhibited low levels of cross-resistance to sethoxydim. On the other hand, two highly sethoxydim resistant populations, F2 and ES4, (with R/S: ~12) were slightly resistant to clodinafop-propargyl. Four of 12 populations (F2, S2, S4 and ES4) always showed high cross-resistance to pinoxaden with  $ED_{50}$  values 12.4- to 27.8-fold greater than the susceptible population. M2, a highly clodinafop-propargyl resistant population, was more sensitive to pinoxaden than the susceptible population suggesting some evidence for the negative cross-resistance to the herbicide. Overall, there was a close similarity between the results of the bioassay and those observed in whole-plant experiment.

Keywords: Avena ludoviciana, Herbicide resistance, Resistance ratio, Seed bioassay, Whole-plant assay.

#### EVALUATING THE RISK OF HERBICIDE RESISTANCE IN WEEDS IN GLYPHOSATE-BASED MAIZE CROPPING SYSTEMS. A SIMULATION STUDY

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The introduction of herbicide-tolerant (HT) genetically-modified (GM) crops has been reported to favour the selection of glyphosate-resistant weeds. When these crops are introduced into cropping systems, they also change agricultural practices other than herbicides, often resulting in simplified rotations and tillage. These changes can mitigate or amplify the risk of herbicide resistance, and thus weed harmfulness for crop production as well as weed contribution to biodiversity. The objective of the present study was to evaluate the risk of herbicide resistance in GMHT maize cropping systems and its impact on weed-related biodiversity and crop production with a model.

We adapted the existing weed dynamics model FLORSYS (Colbach *et al.*, 2014) to simulate the advent and progress of herbicide resistance by mutation, heredity, fitness costs and selection due to glyphosate applications and other cultural practices. Parameters were estimated from literature. A sensitivity analysis was carried out, showing that weed dynamics were very sensitive to mutation rates and to which weed species could become resistant; the effect of selfing rate, fitness cost and glyphosate efficiency was negligible.

Current and prospective maize cropping systems were identified from expert opinion and data bases (Bürger *et al.*) and simulated here. Switching from conventional to GM HT maize rotations multiplied the density of glyphosate-resistant weeds by approximately 5, but resistant weeds only became important when rotations were further simplified to GM HT maize monoculture (x 11) and, particularly, when abandoning all tillage (x 70-100). Conversely, catch crops reduced resistant weeds.

Simulations with and without occurrence of herbicide-resistant weeds were carried out to evaluate the effect of herbicide resistance on weed-related biodiversity and harmfulness. Including herbicide resistance generally improved vegetal biodiversity and trophic resources for birds, carabids and bees as it improved the survival of species hitherto infrequent in the tested cropping systems. Conversely, it usually also increased weed harmfulness (yield loss, harvest contamination).

The present work was financed by INRA, the French project CoSAC (ANR-14-CE18-0007) and the European project AMIGA (FP7-KBBE-2011-5-CP-CSA).

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#### GLYPHOSATE RESISTANCE ON CONYZA SPECIES: MECHANISM AND STANDARDIZATION OF SCREENING TESTS

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Horseweed [*Conyza canadensis* (L.) Cronq.] has been the most frequent weed species to develop resistance to glyphosate in various parts of the world, including Greece. In order to investigate the resistance mechanism, a large number of susceptible (S) and resistant (R) populations were investigated. Expression levels of the *EPSPS* key enzyme and *4 ABC transporter genes* such as *M10*, *M11*, *M7* and *P3* were studied, in a number of contrasting (in terms of resistance level) populations. Screening *in-vivo* test (shikimate analysis) was standardized to eliminate the false positive or negative results. For dose-response experiments, plants at the small rosette stage (8-10 leaves, 20 cm height) were sprayed with glyphosate as previously mentioned, with the following doses: 1/2X, 1X, 2X, 4X, and 8X (where 1X represents the recommended dose of 720 g a.i. ha<sup>-1</sup>). Measurement of the shikimate was done using leaf samples.

For molecular studies, RNA was isolated from leaves, and cDNA was synthesized using standard techniques. Specific primer sets were used to amplify a 1346bp *EPSPS* gene fragment, possibly containing the P106 mutation and to amplify the *P3, M7, M10, M11 ABC- transporter* genes from the resistant and the susceptible biotypes. Relative quantification was performed using the  $\Delta\Delta$ Ct method according to the internal software of the thermal cycler. Relative expression profiles for each target gene were estimated by comparing the expression level of each sample with the expression level in the untreated susceptible control plants (Cs).

The proposed resistance mechanism was not due to a point mutation at codon 106 of *EPSPS* gene that regulates glyphosate metabolism (target-site resistance). Previous studies have also highlighted the importance of the aforementioned genes on glyphosate resistance. In the present study, it was documented a characteristic synchronization pattern of the overexpression of *EPSPS* and *ABC-transporter* genes (particularly *M10* and *M11*) that were involved in the resistance mechanism. This synchronization pattern is characterized by: a. time of induction and period of the overexpression for key genes, and b. the initial glyphosate load

Standardization of the shikimate test, improved (by approximately 30%) the accuracy of the test. Those results together with appropriate methodology will be fully discussed in this paper aiming to provide the necessary tools for management of glyphosate-resistant weeds.

#### IDENTIFICATION OF GENES INVOLVED IN NON-TARGET-SITE BASED RESISTANCE TO ALS INHIBITORS IN *LOLIUM* SP.: A STEP TOWARD THE DEVELOPMENT OF DIAGNOSTIC TOOLS

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Non-target-site-resistance (NTSR) to herbicides is a major cause for chemical control failure in a number of weeds, and is especially widespread and frequent in major grass weeds. In particular, NTSR of grass weeds to herbicides targeting acetolactate-synthase (ALS) is an increasingly challenging issue in Europe. Although several gene families have been shown to be involved in NTSR, the genetic determinants of NTSR are still poorly known. Here, we investigated the transcriptomic bases of constitutive NTSR of the major weed rye-grass to ALS inhibitors. The objective of this study was to identify genes that were differentially expressed in resistant (R) plants compared to sensitive (S) plants in the absence of herbicide application.

(pyroxsulam Usina two maior herbicides applied to control rye-grass and iodosulfuron+mesosulfuron), we implemented a comparative RNA-seq of pools of untreated R or S plants of Lolium sp. from four populations. The expression level of contigs potentially encoding a total of 81,663 predicted peptides was monitored and compared among pools, and 30 candidate NTSR contigs were identified on the basis of their overexpression (at least twice) in each R pool compared to each matching S pool. Candidate contig expression was then measured individually in 247 plants from 17 rye-grass field populations using RT-quantitative PCR. Nineteen contigs were overall up-regulated in many but not all R plants compared to the S plants. Not all contigs were up-regulated in all R plants, and the contigs up-regulated varied with the individual plant. Overall, R plants from populations with the highest frequency of R plants showed candidate contig expression levels higher than plants from populations with lower frequency of R plants, suggesting that herbicide selective pressure gradually selected individuals with higher and higher expression levels for the candidate NTSR contigs investigated. Contig-by-contig expression level analysis did not allow satisfactory discrimination of R plants from S plants. Further analyses indicated that combining the expression levels of all candidate NTSR contigs greatly improved the discrimination of the R plants from the S plants. Also, considering sensitivity of each plant to both ALS inhibitors considered, and not to only one, further improved the identification of plants with NTSR. This suggested that NTSR pathways concerning both herbicides investigated existed.

Our work confirmed that NTSR is under polygenic control, and that NTSR mechanisms vary among individual plants. Several genes are clearly generally necessary to obtain a NTSR phenotype. Accordingly, measuring the expression of a range of genes seems necessary to be able to clearly identify NTSR plants. The direct role of the candidate contigs identified in NTSR needs to be established by functional validation, and the mutations at the root of the differences in expression observed remain to be identified.

#### MECHANISM OF RESISTANCE TO MESOTRIONE IN AN AMARANTHUS RUDIS POPULATION FROM NEBRASKA, USA

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Amaranthus rudis is a troublesome weed in corn and soybean production systems in midwestern USA due in part to its ablity to evolve multiple resistance to key herbicide modes of action including acetolactate synthase, photosystem II, glyphosate, protoporphyrinogene oxidase, auxin and 4-Hydroxyphenylpyruvate dioxygenase (HPPD) herbicides. Here we have investigated the mechanism of resistance to mesotrione, a key component for managing broadleaf weeds in corn, in a multiple herbicide resistant population (NEB) from Nebraska.

NEB showed a 30-fold resistance increase to mesotrione compared to a sensitive population SEN-1 in a whole plant post-emergence dose response test. Sequencing of the whole HPPD gene from 12 each of sensitive and resistance plants did not detect any target site mutations that could be associated with resistance in NEB. Resistance was not due to HPPD gene duplication in resistant plants either as revealed by Q-PCR analyses. Additionally, no difference in mesotrione uptake was detected between NEB and SEN-1. In contrast, higher levels of mesotrione metabolism via 4-hydroxylation of the aryl ring was obsevered in NEB compared to the sensitive population.

This study demonstrates yet another case of non-target-site based resistance to an important class of herbicides in an *Amaranthus rudis* population. The knowledge generated will help to design strategies for managing multiple herbicide resistance in this problematic weed species.

#### HYBRIDIZATION RATES BETWEEN WHEAT (*TRITICUM AESTIVUM*) AND ITS WILD RELATIVE *AEGILOPS TRIUNCIALIS* UNDER REAL FIELD CONDITIONS

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Aegilops triuncialis (L.) is the most worldwide spread species of Aegilops genus, frequent in the Mediterranean area. It is commonly found in many European countries and populations are usually found in proximity to wheat cultivations. The flowering times of wheat and Aegilops can overlap in south European fields. Although invasive, *Ae. triuncialis* is generally not considered weedy in the agronomic sense, but is a troublesome weed in California. It is a colonizing species with the capacity to develop large stands, up to many hectares, that could increase their invasiveness under the selection pressure of the herbicide whether herbicide resistance genes found in wheat can naturally introgress into *A. triuncialis* via hybridization.

The fact that hybrids between wheat and *Ae. triuncialis* can be produced and that they can be partially fertile, after backcrossing to any of the parents or by spontaneous amphiploidy (Loureiro *et al.* 2009), indicates that wheat genes can be introgressed to the wild *Ae. triuncialis* populations, as has been recently shown using wheat diagnostic alleles (Parisod *et al.* 2013). The transference of herbicide resistance genes from wheat to *Aegilops cylindrica* (Host), a noxious weed in wheat-producing areas of the western United States, has been detected in the field which could compromise its control by herbicides.

During the field surveys conducted by our team for collecting *Ae. triuncialis* populations, two natural hybrids were found in *Ae. triuncialis* stands growing in close proximity to wheat fields. Although hybridization can occur, there is no information available on the frequency of its occurrence under natural conditions. The frequency at which *Ae. triuncialis* hybridize with wheat where both species co-occur was estimated in the Meseta Central, Spain's central plateau where wheat is the major crop. A total of more than 70,000 seeds from 54 *Ae. triuncialis* populations growing in wheat close proximity were collected and grown in the greenhouse. Hybrids were identified by their intermediate spike morphology, similar to that of the hybrids obtained by hand crossing. The hybridity of the plants was confirmed by their chromosome number (2n=35 chromosomes). Hybrids were found in 3 of the 54 populations at rates up to 0.24%. The potential risks associated with natural hybridization in the context of transgenic wheat cultivation are discussed. Data presented provide a new step in acquiring the knowledge on the potential *Ae. triuncialis*-wheat herbicide resistance transference, basic for risk assessment purposes.

# CHLORSULFURON RESISTANCE IN *LOLIUM RIGIDUM* GAUD. IN WINTER CEREAL FIELDS IN SPAIN: EVOLUTION OF RESISTANCE 10 YEARS AFTER

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Lolium rigidum Gaud. is the most prevalent and damaging grass weed of winter cereals in Spain. *L. rigidum* infestations are frequently treated with herbicides and, consequently, populations have evolved resistance. In 2012-2013 a random survey was conducted across cereal cropping areas of the Castilla-León and Cataluña regions to establish the distribution and frequency of herbicide resistance in *L. rigidum* populations to the ALS-inhibiting herbicide chlorsulfuron, commonly used for rigid ryegrass control in Spain. The results of this survey were compared with the results of a previous survey conducted in 2000-2002.

In a plant herbicide screening assay conducted 10-12 years ago, chlorsulfuron applied at the field dose of 15 g a.i. ha<sup>-1</sup> provided a good control of ryegrass populations from Castilla-León, where 89.5% of the populations were susceptible, 9.3% showed certain degree of herbicide resistance and only 1.2% of populations were detected as being resistant. In Cataluña, although no herbicide resistant populations were found, 60% of the populations displayed some level of resistance and 40% were susceptible (Loureiro *et al.* 2010). At present, the scenario of chlorsulfuron resistance is quite different. The efficacy of this herbicide has decreased significantly and resistance levels have increased since the last survey: 28.5% of the populations of Castilla-León were found to be resistant, while 57% showed intermediate levels of resistance and only a 14.5% were susceptible. The situation in Cataluña was more dramatic, with no susceptible populations and 33% of resistant populations.

This weed survey shows that a high occurrence of resistance to chlorsulfuron is now present in rigid ryegrass populations from cereal cropping regions in Spain. Fortunately, there are still active ingredients effective to control these resistant populations. It will thus be necessary to diversify herbicide use by rotating herbicides with different modes of action, by alternating crops and by implementing diversified cropping programs, and to introduce integrated weed management programs as diverse as possible. This will allow us to diminish the levels of resistance and to prevent the development of new ones, while preserving both fields and herbicides.

# OCCURRENCE AND SPREAD OF RESISTANCE OF COMMON GROUNDSEL (SENECIO VULGARIS) TO ACETOLACTATE SYNTHASE (ALS) INHIBITORS

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Common groundsel is a tetraploid, highly selfing broadleaved species that is a widespread weed present in many habitats, from arable fields to pavements. Its ability to produce up to thousands of seeds, and the aptitude of these seeds to be disseminated by wind, water or machinery and to germinate at any time make this plant a potentially problematic weed. Following recurrent failure of groundsel control with ALS inhibitors over several years, occurrence of resistance was investigated in 2013 in two nearby wheat fields in Brittany (France). Bioassays confirmed resistance of common groundsel to eight herbicides in four different chemical families of ALS inhibitors. While resistance levels were highest to sulfonylurea herbicides (flazasulfuron, metsulfuron, iodosulfuron+mesosulfuron, prosulfuron and tribenuron), efficacy of herbicides in the imidazolinone (imazamox), triazolopyrimidine (florasulam) and sulfonylaminotriazolinone (thiencarbazone) families was also affected.

Target site resistance (TSR), which is the most commonly reported mechanism that confers resistance to ALS inhibitors in broadleaved weeds, was investigated. Sequencing the two homeolog genes encoding ALS in common groundsel (ALS1 and ALS2) revealed the presence of an amino-acid substitution at codon 197 in ALS1 in all resistant plants sequenced (proline-to-leucine). Interestingly, plants from each field with a same genotype at ALS could show different resistance levels to a same herbicide, suggesting non-target-site based resistance mechanisms could be present in common groundsel together with TSR. This requires further investigation.

We developed a single dCAPS PCR test designed to detect all possible amino-acid substitutions at codon 197 in both ALS1 and ALS2. The dCAPS assay was used to investigate the propagation of ALS-based resistance in common groundsel from the two fields were resistance was first detected. In 2014, common groundsel plants were sampled in 33 randomly selected fields (50 plants per field) located 0.2 to 15 km from the 2 fields where resistance was initially detected. The presence of the mutation was detected in 31 of the 33 fields, with a frequency of mutant plants ranging from 2 to 100%. In 2015, a second sampling was performed at a broader scale (70 fields, 10 to 75 km from the 2 fields). Mutations were detected in 49 of the 70 fields (2.2 to 97.8% mutant plants) and up to a distance of 62 km from the two originating fields. From our results, it is clear that herbicide resistance readily and rapidly propagates over substantial distances in common groundsel.

This study demonstrated evolution and long-range dissemination of herbicide resistance from a single TSR appearance event in common groundsel. Resistance of common groundsel is thus not a single grower's problem: after it has been selected for in one field, resistance rapidly concerns a large number of fields and farms. Occurrence of common groundsel in non-arable areas (roadsides, wastelands...) from which seeds can contaminate arable fields further complicates resistance management. Resistance of common groundsel should therefore be managed by an approach similar to that implemented for air-borne fungal diseases, i.e., by a concerted action involving growers as well as a holders and managers of non-arable areas.

# FIRST EUROPEAN CASES OF SORGHUM HALEPENSE RESISTANT TO ALS INHIBITORS: RESISTANCE PATTERNS AND MECHANISMS

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Acetolactate synthase (ALS) inhibiting herbicides are among the most widely-used herbicides in Europe and worldwide, and have proved to be prone to select for resistant populations. The number of resistant (R) cases reported for spring-summer grass weeds is increasing. A few cases of *Sorghum halepense* (L.) Pers. (johnsongrass) resistant to ALS inhibitors have been reported from the USA, Chile, Mexico and, in Europe, from Italy. *S. halepense* is a geophyte that reproduces abundantly by both rhizomes and seeds. A complaint monitoring was done in Italy and Hungary where poor control of johnsongrass had been reported. Repeated greenhouse screenings with two doses of nicosulfuron (1x=60 g a.i. L<sup>-1</sup> and 3x) proved that eighteen Hungarian and two Italian populations were resistant or highly resistant to the ALS inhibitor nicosulfuron. Populations showed different resistance patterns.

To better investigate the level of resistance and elucidate the mechanisms underlying the resistance, four populations, two Hungarian and two Italian, were chosen among those resistant. A dose-response pot experiment testing four different ALS-inhibiting herbicides (foramsulfuron, nicosulfuron, imazamox and bispyribac-Na) was conducted on these R populations along with two susceptible controls (one Hungarian and one Italian). A purified seed stock from resistant plants was used to determine the presence of target-site and non-target-site resistance mechanism(s) through: (1) in vitro ALS enzyme bioassay (2) treatment with metabolic inhibitor (i.e. malathion 2 kg ha<sup>-1</sup>) to investigate the possible presence of enhanced metabolism; (3) molecular analysis to detect the mutant ALS alleles.

The two Italian populations (07-13 and 12-54A) resulted as being highly cross-resistant to all the ALS inhibitors tested. The mutant ALS allele Leu<sub>574</sub> was identified in all plants of these populations. But the plant responses to the herbicide treatment with malathion gave different results: plants of population 12-54A showed a significant decrease in plant survival and fresh weight with respect to plants of population 07-13. This suggests that an enhanced metabolism may also be involved in the resistance. The Hungarian populations (08-16 and 12-49a) proved to be controlled by imazamox while they were resistant (with different levels) to sulfonylureas and bispyribac-Na. Plants from population 08-16 presented the mutant allele Glu<sub>376</sub>. ALS enzyme bioassay and treatment with malathion confirmed that in this population the only resistance mechanism involved is target-site mediated, whereas the results indicated that in plants of population 12-49a the resistance is very likely due to enhanced metabolism of P450 enzymes. No mutations have so far been detected in plants of population 12-49a, but experiments are still ongoing.

The results clearly suggest that resistance to ALS inhibitors in *S.halepense* involves more than one resistance mechanism and both mechanisms (target-site and non-target-site) can likely occur in the same plant. This is a troublesome situation in maize because no alternative herbicides are available to control *S. halepense* originated from rhizomes and pre-emergence herbicides is the only alternative to control *S. halepense* from seeds.

#### IDENTIFICATION AND DISTRIBUTION OF ALS RESISTANT SORGHUM HALEPENSE POPULATIONS IN SERBIA

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Sorghum halepense (L.) Pers. is one of the most troublesome weeds in Serbian field crops production. Recently, resistance of *S. halepense* to some sulfonylurea herbicides has been confirmed in Italy and Hungary. Continuous application and the poor efficiency of some sulfonylurea herbicides applied in maize, indicate that the same phenomenon exists in some regions of Serbia. The aim of this study was to determine if and where resistance of *S. halepense* to the acetolactate synthase (ALS)-inhibiting herbicides occurred in Serbia.

In 2013 and 2014 a total of 25 S. halepense populations suspected for resistance to ALSinhibiting herbicides were sampled from maize fields in Southern Banat, Mačva, Stig and Northern Bačka region of Serbia. Suspected resistant population from S. Banat (R) and susceptible population from vicinity of Novi Sad (S) were used for herbicide dose response trials. Whole plant bioassays were carried out in greenhouse conditions (28/22±3°C day/night temperatures with a 16h photoperiod) designed as a randomized randomized complete block design with 4 replications (8 plants per replication) and repeated. Herbicides nicosulfuron (0.23 - 7700 g a.i. ha<sup>-1</sup>), rimsulfuron (0.16-2560 g a.i. ha<sup>-1</sup>), imazamox (0.63-2560 g a.i. ha<sup>-1</sup>), pyroxsulam (1.5-12000 g a.i. ha<sup>-1</sup>), propoxycarbazone-Na (0.33-5400 g a.i. ha<sup>-1</sup>) and cycloxydim (0.78-200 g a.i. ha<sup>-1</sup>) were applied at two-three leaves growth stage of S. halepense seedlings, with portable sprayer, flat fan nozzle XR11002 and 280 L/ha of water. Shoot fresh and dry weight per pot and visual growth reduction in relation to the untreated control were recorded 14 days after the herbicides' applications, respectively. Using the "R" software and *drc* package, GR<sub>50</sub> and resistance index (RI) were determined for the susceptible and suspected resistant populations. For testing of populations from other locations to confirm ALS-resistance, we used a nicosulfuron at recommended rates (60 g a.i. ha<sup>-1</sup>) and 10-fold increased rates.

Results from whole plant bioassays confirmed that tested populations from Southern Banat, Mačva (close to river Drina), Stig and Northern Bačka region (close to Hungarian border) are resistant to nicosulfuron, and R population from S. Banat was highly cross-resistant to all tested ALS inhibitors from four different chemical groups. The results showed a high level of resistance of the population from S. Banat on whole plant level (fresh shoot weight) to nicosulfuron and rimsulfuron (RI>2000), and imazamox (RI>800). In contrast to this, ALS-resistant populations were susceptible to cycloxydim, which could be very important chemical option in the management of ALS-resistant *S. halepense* from rhizome in cycloxydim-tolerant maize. We estimate that the ALS-resistant populations of *S. halepense* are infesting at least 50,000 ha of crop fields in Serbia, and suggest it is necessary to implement urgently the measures for resistance management.

# TRACING BIOTYPES OF TURNIP WEED (*RAPISTRUM RUGOSUM* L.) RESISTANT TO TRIBENURON-METHYL IN WHEAT FIELDS OF IRAN

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A survey of 100 wheat fields was conducted across Golestan province-Iran in spring 2012-2013 to determine the ALS-inhibitor resistance in *Rapistrum rugosum* L. Seeds were collected from fields and planted into the greenhouse. The study was carried out in two steps including screening turnip weed biotypes with discriminating dose and determining resistance factor by dose response experiments in the greenhouse. After screening tests for 20 suspected resistant biotypes collected during these two years, 7 biotypes were recognized as resistant (R). In the whole plant studies, experiments were arranged in a completely randomized design with three replications. After breaking seed dormancy, five seedlings from R biotypes along with a susceptible biotype (S), from regions with no chemical control record, at the cotyledon stage were transplanted into suitable pots. The plants were sub-irrigated and grown to the four-leaf stage under supplemented natural light in the greenhouse. In four-leaf stage, a foliar application of tribenuron-methyl for S and R biotypes was made, equivalent to 0, 0.25, 0.5, 1, 2, 4, 8, 16 and 32X the regular field use rate or 0, 3.75, 7.5, 15, 30, 60, 120, 240 and 480 gr a.i. ha<sup>-1</sup>. Four weeks after spraying, plants were harvested and measured dry above-ground weight. The sigmoidal model used to fit the weed control data allowed the estimation of resistance index.

The whole-plant bioassay confirmed that the R biotypes were resistant to tribenuron-methyl, the calculated  $GR_{50}$  value for the R biotype treated with tribenuron-methyl show this biotypes were 1.8 to 6.4 fold resistant to tribenuron-methyl, at the whole plant level compared with the susceptible biotype.

Keywords: tribenuron-methyl, *Rapistrum rugosum* L., whole plant experiments, herbicide resistance

# INHERITANCE OF ALS HERBICIDE RESISTANCE IN TRIPLEUROSPERMUM PERFORATUM

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In a resistance survey conducted in Germany in 2011 by the Julius Kühn-Institut and EpiGene, several biotypes of *Tripleurospermum perforatum* with resistance to the active substance tribenuron (ALS inhibitor) were identified. During this survey, resistant biotypes were characterised regarding their resistance level and cross-resistance pattern as well as the underlying molecular basis of resistance.

For *T. perforatum*, no information is so far available on the inheritance of the resistance trait and the possibility of a transfer of the resistance trait from resistant to sensitive plants via pollen. As the species *T. perforatum* is known to be insect pollinated, a possible transfer of the resistance trait via the pollen can be assumed.

To test this hypothesis, a semi-field trial was conducted in 2013 and 2014. Ten plants from a resistant biotype (R) with an identified target site resistance (Pro-197Gln) were used. These plants were heterozygous for the respective mutation. R plants were pair-crossed to one plant of a susceptible reference population (S) to produce a total of 10 pair crosses that generated 20 F1 populations. Testing for ploidy level of R and S plants showed that all 20 plants used in the experiment were diploid. Plants grown from the F1 seeds were then analysed for their herbicide sensitivity in dose-response trials using the active ingredient tribenuron and the sensitivity of the F1 generation was compared to sensitivity level of the R and S parent biotypes. In addition, plants surviving herbicide application were tested for target site resistance at position 197 using pyrosequencing. This allowed an identification of the number of resistant alleles in each plant. The null expectation for inheritance of the R trait was a Mendelian segregation of one dominant allele.

Results show that a transfer of the resistant trait from R to S plants occurred but resistance level differed between F1 populations. Additionally, not all F1 plants appeared to carry the Pro197Ser substitution. These variations are believed to be due to fact that all R parent plants were heterozygous for the respective mutation. In general, results indicate that inheritance of tribenuron resistance in R biotype is incompletely dominant.

#### DETERMINATION OF ALS HERBICIDES RESISTANCE BIOTYPES OF GALIUM APARINE L. (CATCHWEED BEDSTRAW) AND BIFORA RADIANS BIEB. (BIFRA) IN WHEAT FIELDS BY BIOASSAY METHODS

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Wheat is the world's most widely grown cereal crop and is a food staple for half of the world's population. Weeds are one of the most important yield limiting factors in wheat which makes effective weed control an integral part of production. *Galium* spp. and *Bifora radians* Bieb. are annual and very competitive troublesome broadleaved weeds dispersed throughout Eurasia and North America. They can cause severe problems in a wide range of climate and habitat but is mainly problematic in winter wheat fields and other winter sown crops.

Due to intensive herbicide use in wheat growing areas in Turkey resistance problems occur in some important weed species. There are some observations concerning the efficacy failures of some ALS inhibitor herbicides on some important weed species, such as Galium aparine and Bifora radians and we received complaints from wheat growers that this species control was reduced when most sulfonylurea herbicides were applied. Studiedactive ingredientsincluding Amidosulfuron+iodosulfuron-methyl-sodium+mefenpyr-diethyl (Safener), Aminopyralid+Florasulam, Cyclosulfamuron, Dicamba+Triasulfuron, Flumetsulam+Florasulam, Mesosulfuron-methyl+iodosulfuron-methyl-sodium, Metosulam+Ethylhexylester, Metsulfuronmethyl, Pyroxsulam+Cloquintocet-mexyl (Safener), Terbutryn+Triasulfuron, Thifensulfuronmethyl+Tribenuron-methyl, Tribenuron-methyl are currently registered against to these species. There is no information in the scientific literature concerning with Galium spp. and Bifora radians response to these herbicides and their level of resistance.

With this aim weed seeds will be collected from different wheat growing provinces in Central Anatolia and Black Sea Region of Turkey, in total from 1371 different wheat fields. After initial screening for resistance in pot experiments, biotypes that are supposed to be resistant will be submitted to bioassays to determine the resistance grades. Some accession exhibited the highest survival to treatment with field rates of these herbicides. The Weibull dose-response curve, an asymmetric sigmoid curve, was fitted to data to obtain ED<sub>90</sub> in R program. Twenty-three of *Galium* spp. and twenty-two of *Bifora radians* accessions tested were not controlled by ALS inhibitors and these were accepted as a resistant population.

The authors thank The Scientific and Technological Research Council of Turkey (TUBITAK) and Ondokuz Mayıs University for supporting the Project (Numbers TOVAG 1090521 and PYO.ZRT.1905.12.002).

# MICROEVOLUTION OF ALS INHIBITOR HERBICIDES RESISTANCE IN APERA SPICA-VENTI (L.) BEAUV.

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Apera spica-venti is the most important weed in Eastern Central Europe and an increasing number of populations are resistant to ALS inhibitor herbicides and no new herbicide mode of action will become available for the next decade. An evolutionary directed weed management strategy would help limit the evolution of herbicide resistance, secure higher crop yields and comply with pesticide use regulations. However, better knowledge of the early micro-evolutionary changes involved in herbicide resistance is needed. The aim of this project is to determine the correlation between the frequency of expressed target site (TSR) and non-target site resistance (NTSR) genes involved in resistance to ALS inhibitor herbicides and the fitness of *A. spica-venti* populations showing a gradient of variations in resistance phenotype.

In order to determine the spatial gradient of variations of populations showing an ALS inhibitor herbicide resistant phenotype and to quantify the extent of multiple resistance, dose responses experiments were conducted with iodosulfuron (ALS inhibitor), fenoxaprop-P (ACCase inhibitor), and prosulfocarb (lipid synthesis inhibitor) on eight populations from six neighbouring fields in Denmark. Each experiment was replicated three times. Populations were analysed for TSR mutations at four positions in the ALS gene, five TSR positions in the ACCase gene, and for metabolic NTSR activity. A transcriptome was assembled for *A. spica-venti* from seven different plant tissues and growth stages from a mixture of ALS susceptible biotypes.

Our results show multiple resistance of ALS resistant biotypes with ACCase but with no resistance to prosulfocarb. Based on ED50 values, there was a significant spatial gradient pattern of resistance where resistant populations form an 'epicenter' of resistance in one particular field. Resistance to ALS inhibitor herbicides was due to metabolic NTSR while resistance to ACCase inhibitor was due to the level of both NTSR and TSR at position 1781. The herbicide resistance response was quantitative for both herbicides, which is congruent with an NTSR mechanism. Several cytochrome P450 monooxygenase genes endowing herbicide resistance have been identified as predominantly expressed in leaves and root of mature plants but not in germinated seeds, thus explaining the susceptibility to prosulfocarb. Our study demonstrates the importance of NTSR mechanisms in the early evolution of ALS inhibitor herbicide resistance in *A. spica-venti*.

# GROWTH AND IMPACT OF HERBICIDE SENSITIVE AND MULTIPLE RESISTANT ALOPECURUS MYOSUROIDES IN WINTER WHEAT

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Blackgrass (*Alopecurus myosuroides* HUDS.) is a troublesome weed of winter crops in Europe. Herbicides inhibiting acetolactate synthase (ALS) and acetyl-coenzyme A carboxylase (ACCase) have been widely used for its control in the last decades. As a consequence, various populations of *A. myosuroides* evolved herbicide resistance. In Germany, multiple resistance to ACCase and ALS inhibitors was frequently observed in *A. myosuroides*. The resistance is often based on non-target-site resistance (NTSR) mechanisms.

In this study, we examined the efficacy of fenoxaprop-P-ethyl and flupyrsulfuron-methyl on sensitive (S) and multiple resistant (R, NTSR) *A. myosuroides* in winter wheat. Furthermore, biomass and seed production were investigated, in the absence of herbicides, to see if there are differences between the populations.

In 2012/2013, field experiments were conducted at the Ihinger Hof research station near Stuttgart in Germany. Experimental factors were *A. myosuroides* (no *A. myosuroides* / S / R) and treatment (no herbicide / fenoxaprop-P-ethyl / flupyrsulfuron-methyl). Concurrently with the emergence of wheat, 60 *A. myosuroides* plants m<sup>-2</sup> were transplanted into the field. At leaf stage 1-3 fenoxaprop-P-ethyl and flupyrsulfuron-methyl were applied.

In the absence of herbicides, no significant differences in the number of ears m<sup>-2</sup> were detected between S (380 ears m<sup>-2</sup>) and R (375 ears m<sup>-2</sup>) plants. Significant differences were observed with regard to seed production. The S population developed significantly more seeds (80 g m<sup>-2</sup>) than the R population (62 g m<sup>-2</sup>) in the absence of herbicides. Herbicide efficacy was significantly higher for the S than for the R population. S plants could be controlled by 85% with fenoxaprop-P-ethyl and by 83% with flupyrsulfuron-methyl. The R population could be controlled by 17% with fenoxaprop-P-ethyl and by 14% with flupyrsulfuron-methyl. The highest number of plant was detected in the absence of herbicides. Statistically the same number was observed for R plants treated with fenoxaprop-P-ethyl (289 ears m<sup>-2</sup>) and flupyrsulfuron-methyl (283 ears m<sup>-2</sup>). A significantly lower number of ears m<sup>-2</sup> was registered for S plants treated with fenoxaprop-P-ethyl (14 ears m<sup>-2</sup>) and flupyrsulfuron-methyl influenced by the experimental factor *A. myosuroides*: 7.2, 6.1 and 5.9 t ha<sup>-1</sup>t in plots without *A. myosuroides*, with S and with R *A. myosuroides*, respectively.

Results illustrated the problem of non-target site resistance in agriculture. Due to a similar metabolic pathway the efficacy of active ingredients with different chemistries can be reduced. The investigated population was highly resistant to herbicides with different herbicide modes of action. Although herbicides were applied for its control, the number of ears m<sup>-2</sup> was statistically the same as in the untreated plots. In the absence of herbicide, the R *A. myosuroides population* has been shown to develop a significantly lower number of seeds than the S population. Nevertheless the R seeds remain in the soil and complicate *A. myosuroides* control in the subsequent years.

#### A LONG TERM STUDY ON SPATIAL AND TEMPORAL DEVELOPMENT OF ACCASE AND ALS INHIBITOR RESISTANCE IN BLACKGRASS (*ALOPECURUS MYOSUROIDES* HUDS.). REVIEW OF A THREE YEAR PERIOD IN NEIGHBORING FIELDS IN GERMANY

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The repetitive use and the single reliance of a limited number of different Modes of Action (MoA) has unquestionably led to an increasing development of herbicide resistance in key weeds in several key cropping areas worldwide including Europe.

So far research on herbicide resistance development has been limited in space or/and time. However, no study has systematically surveyed more than 100 fields closely located during a period of more than 3 years. Our objective is to show how unique and patchy herbicide resistance can occur and furthermore how farming practices act as a driver in the selection process. The objective is to reveal the evolution of different resistance clusters and what are the main contributing factors.

Our work started with three initial fields approximately 10 km apart from each other representing the starting point for three locations. Each field had a distinctive ALS-resistance status for blackgrass (*Alopecurus myosuroides* Huds.). Blackgrass occurred in all fields prior herbicide application by the farmer. In each location, about 40 neighboring fields in close proximity to the original field have been sampled annually since 2011. Seed samples were drawn from these fields and infestation was rated. The seeds were then tested in the greenhouse with commercially available ACCase and ALS inhibitors and survivors were analyzed in the laboratory for target sitemutations conferring resistance to ALS and ACCase inhibitors (TSR) known for blackgrass. The whole study is planned for the period 2011-2016 and we report here the data so far obtained for the period of 2011-2013. Resistance evolution was analyzed in the context of farming practice using detailed field history.

The majority of the sampled fields showed agronomic relevant resistance to at least one ACCase inhibitor. Agronomical relevant ALS inhibitor resistance was only observed in some fields with significant variation between years. Interestingly to note is the dominating occurrence of Pro197 mutation in one location while Thr574 was almost exclusively occurring in the other. The third location does not show agronomic relevant ALS resistance. Plants with resistance to ALS inhibitor, mesosulfuron-methyl, involving only enhanced metabolic resistance (EMR) were observed in rare cases. A resistance structure consisting out of field infestation ratings, greenhouse bioassay data and frequency of target site mutations was determined for every field. The analysis of these findings strongly suggested that spatial and temporal development of resistance are mostly independent between neighboring fields. Including detailed field history, results are discussed with regard to the agronomic practices such as soil management, crop rotation and/or herbicide regime. These results show how Integrated Weed Management (IWM) tools can be used to keep the population pressure low.

# GENETIC DIVERSITY OF RESISTANT AND SUSCEPTIBLE GLYPHOSATE POPULATIONS OF LEPTOCHLOA VIRGATA COLLECTED IN CITRUS ORCHARDS IN MEXICO

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Citrus production in the state of Veracruz is one of the most important agricultural activities, mainly producing persian limes (*Citrus latifolia*) and orange (*Citrus sinensis*). The presence of weeds is the main limiting factor of production in these crops. The weed management in the municipalities of Martinez de la Torre and Cuitláhuac in the last 15 years has been based mainly on the application of glyphosate 4-5 times per year. Due to the agronomic and economic problems caused by *Leptochloa virgata* (Lam.) Gray in these crops, is great importance study the status, distribution and characterization of this species in this region. In this study we characterized the efficacy of glyphosate and the genetic diversity of 17 biotypes of *L. virgate* suspected glyphosate resistant collected in Martinez de la Torre and Cuitlahuac, Veracruz using ISSR markers.

Results show different levels of resistance to glyphosate in those populations: 14 populations showed a resistance factor higher than 2 and a maximum accumulation of shikimic acid of 227 ± 54 mg g<sup>-1</sup> fresh weight in resistant populations at 96 hours after treatment (HAT). Also 2 susceptible populations were identified with a resistance factor of 1.1 (LV11) and 1.2 (LV9) compared with the population sensible (LVS), and with an accumulation of shikimic acid on average 15 times higher than the resistant populations at 96 HAT. Spray retention assay indicated that the leaf retention is not an important factor for glyphosate resistance in this species. To reveal the effects of herbicide selection on genetic diversity in of L. virgata, those populations (3 glyphosate-susceptible and 14 glyphosate-resistant) were analyzed by using 40 polymorphic intersimple sequence repeat loci (ISSR). Cluster and principal coordinate analyses (PCO) grouped individuals mostly by response to glyphosate irrespective of response to localized geographic region. Analysis of molecular variance (AMOVA) was carried out based on the response to the herbicides. The within population contribution towards total variation was always higher than among groups and among populations. High levels of within population genetic variation under the sustained glyphosate selection pressure could be responsible for rapid development of herbicide resistance and the variation in levels of resistance found in those Mexican populations.

Keywords: glyphosate, resistance, ISSR markers, resistance factor.

# GENETIC CHARACTERIZATION OF EPSPS FROM CONYZA CANADENSIS RESISTANT TO GLYPHOSATE

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A resistant population of *Conyza canadensis* (L.) Cronq., (B15), was selected after several years of application of glyphosate from an intensive olive grove in Alentejo (south of Portugal).

In order to study the hypothesis of target site resistance related to 5-enolpyruvylshikimate-3phosphate synthase (EPSPS), shikimate leaf disc assay, sequencing of EPSPS gene and expression levels of the gene encoding this enzyme with real-time PCR were carried out.

After being exposed to glyphosate the population B15 showed a lower accumulation of shikimate compared to a reference susceptible population (B). The sequencing of cDNA encoding EPSPS revealed two single nucleotide polymorphisms but both correspond to silent mutations. The potential mutations, responsible for glyphosate resistance, i.e. Gly101Ala; Thr102lle; Pro106Ser/Thr/Gly/Cys/Ala/lle/Val/Met/Leu, Pro106Ser/Thr/Ala/Leu; Gly144Asp/Asn and Ala192Thr were not present. The analysis of gene expression also revealed no differences in expression levels of this enzyme, 24-h after glyphosate application.

These results suggest that the studied population of *C. canadensis* has no EPSPS enzyme mutation or over expression as a mechanism of resistance to glyphosate. Further studies on glyphosate translocation are in course.

Keywords: resistance, target-site, EPSPS, shikimate, real-time PCR.

# GIANT RAGWEED RESISTANCE TO GLYPHOSATE IN NEBRASKA

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Extensive use of glyphosate and Roundup Ready crops has changed farming practices over the last 15 years. Repeated use of glyphosate on over 100 million hectares has developed glyphosate resistance in 13 weed species in the United States. The current suspected glyphosate resistant (GR) giant ragweed population was found in a corn and soybean production system with history of glyphosate use for weed management in David City, NE. Therefore, field experiments were conducted in 2012 and 2013 to determine the level of glyphosate resistance in the suspected GR giant ragweed population in David City, NE.

The experiments were conducted twice with four replications. Trial by treatment interactions was not significant therefore; data were combined over experimental runs and years. Weed control was assessed visually at 7, 14, and 21 DAT, and dry matter data was recorded. Dose response studies were conducted with five glyphosate rates (0, 1X, 4X, 8X, and 16X of label rates) applied postemergence at two application timings (10 and 20 cm). Glyphosate resistance was determined by the ED<sub>80</sub> and ED<sub>90</sub> values of the population. The estimated level of glyphosate resistance based on ED<sub>90</sub> values at 21 DAT for 10 and 20 cm tall giant ragweed was 14X and 36X, respectively. To achieve 90% control of this population, at least 14 times the label use-rate (1060 g ai/ha) was needed, indicating that the suspected giant ragweed population was glyphosate-resistant.

# WATERHEMP RESISTANCE TO POST-EMERGENT APPLICATION OF HPPD HERBICIDES

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Crop production systems in the United States are facing a major challenge with increasing number of weed species evolving resistance to herbicides. In 2009, waterhemp (*Amaranthus tuberculatus syn. rudis*) biotypes resistant to HPPD-inhibiting herbicides were first reported in Iowa and Illionois. Waterhemp has been reported to be resistant to three mechanism of actions in Nebraska; PSII, HPPD, and synthetic auxins-inhibiting herbicides. Field studies were initiated in 2012 and 2013 to determine level of waterhemp resistance to post-emergent applications of HPPD-inhibiting herbicides in a population reported from Nebraska.

A total of five doses (0, 1X, 2X, 4X, and 8X) of suggested label rates of mesotrione, tembotrione, and topramezone were applied at two application timings (8 and 15 cm). Weed control was visually evaluated weekly until 26 DAT, and weed dry matter was recorded. Based on visual injury and dry matter reduction, dose response analysis was performed to determine ED<sub>50</sub>, ED<sub>60</sub>, and ED<sub>80</sub> values for control of 8 and 15 cm tall waterhemp with mesotrione, tembotrione, and topramezone was 13, 10, and 7 times the label rate, respectively. While levels of resistance to tembotrione and topramezone were not as high as mesotrione, the population was confirmed to be resistant. The use-pattern of HPPD herbicides should be carefully managed and an integrated weed management plan involving tillage and multiple mechanism of actions should be utilized.
## **RESISTANCE STUDY OF (SPANISH) PAPAVER RHOEAS (BIOTYPES) TO BROMOXYNIL**

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Papaver rhoeas is a major weed of cereal crops in southern Europe. Its competitive nature (that can decrease wheat yields up to 32%), makes it especially noxious in winter wheat. It is an increasing problem due to the appearance of herbicide resistant biotypes to synthetic auxins and/or to sulfonylureas. Poor control of corn poppy in Spain with 2,4-D was first reported in 1992 and a biotype resistant to both 2,4-D and tribenuron was reported in 1998. Recently failures of corn poppy control with mixtures containing bromoxynil, have been reported from wheat crops in the north of Spain. The objectives of the current research are to study the effect of bromoxinil in these biotypes, confirm the resistance to ALS inhibitors and auxinic herbicides and identify the mechanism of resistance to ALS inhibitors.

Seeds from two fields where bromoxynil mixtures had failed (B-0313 and B-0413) together with three susceptible populations (AL-S13, BR-S13 and HR-S13) were collected in order to conduct whole plant dose response assays. Bromoxynil was sprayed in three different stages: 3 cm, 5-6 cm (field recommended stage) and 10 cm rosette stage. 2,4-D and Tribenuron-methyl were sprayed at 5-6 cm rosette stage. Four weeks after treatment plants were harvested (above ground) and the fresh weight was measured. The  $GR_{50}$  for the fresh weight was calculated using non linear regression analysis, and resistance factors (RF) from  $GR_{50}$  values ( $GR_{50}R/GR_{50}S$ ).

B-0313 and B-0413 were resistance to tribenuron-methyl with RF of 39.8 and 92.7, respectively, and to 2,4-D, with RF of 17.6 and 3.2, respectively. In the first phenological stage bromoxynil controlled both populations and in the second one the RF were 2.62 and 1.37, respectively. These values increased in the third phenology, 4.82 and 2.94.

In the next season this trial is going to be repeated with these and new biotypes that also showed lack of control with bromoxynil.

## DISCOVERING THE MECHANISM OF ENHANCED METABOLISM IN FLUFENACET RESISTANT RYEGRASS (*LOLIUM* SPP.)

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The control of heavy grass weed infestations in cereals increasingly relies on applications of residual pre-emergence herbicides in a number of cropping areas worldwide. Inhibitors of Very Long Chain Fatty Acid synthesis (VLCFAs, HRAC mode of action classification: K3) including flufenacet based products are key herbicides which offer an alternative mode of action to those of post-emergence herbicides for which resistance can be observed.

Flufenacet resistance in ryegrass (*Lolium* spp.) was documented in the Northwestern cereal growing area of the United States. One population (Walla Walla) offers an ideal plant model to discover the resistance mechanism of this type of herbicide. An Australian ryegrass population (VLR69) showing high metabolic resistance to several herbicides was used for comparison as well as two known sensitive populations. The level of flufenacet resistance was determined for these four ryegrass populations in the greenhouse with dose-response bioassays. Flufenacet metabolism was examined in a time course experiment by application of <sup>14</sup>C-radiolabelled flufenacet on weed seedlings and HPLC analysis. In a third experiment inhibitors of known enzymes involved in xenobiotic detoxification, were studied using a similar approach.

Seedlings of the flufenacet resistant population Walla Walla survived a multiple of the recommended field rate (10x and higher) whereas a shift in sensitivity is consistently observed with VLR69. The level of resistance detected in the greenhouse correlated for the four populations tested with the metabolism rate observed by HPLC analysis. Differences in flufenacet metabolism were particularly high within the first hours after application and could be significantly reduced by application of the glutathione-S-transferase (GST) inhibitor NBD-CI before the herbicide treatment. This strongly suggests that 1.) a quick detoxification response to the herbicide treatment and 2.) the activity of GSTs plays a key role in the flufenacet resistance of these ryegrass populations.

The elucidation of the mechanisms involved in flufenacet resistance will contribute to understanding the development of resistance in field populations and will provide tools for early diagnostics in order to adapt the weed management strategies.

## ANALYSIS OF CANDIDATE GENE EXPRESSION IN BLACKGRASS POPULATIONS EXHIBITING DIFFERENT DEGREES OF METABOLIC RESISTANCE

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Genome-wide gene expression patterns were determined by means of a transcriptomics approach in green leaves of six well characterized multiple herbicide resistant blackgrass (*Alopecurus myosuroides*, Huds.) populations showing different resistance patterns to herbicides with different modes of action in order to identify candidate genes for metabolic herbicide resistance. Prior to analysis, a reference transcriptome was established and the resistant plants were checked for the absence of known target site mutations for ALS and ACCase. Comparison of transcript profiles between sensitive wild type and resistant populations revealed several genes which showed significant higher expression levels in resistant than wild type plants under control conditions as well as after herbicide treatment which was confirmed by quantitative PCR.

In a second step progenies which derived from single plant crosses of sensitive wild type plants with selected individuals derived from the six metabolic resistant blackgrass populations were analysed. Segregating F2-populations obtained were phenotypically assessed for resistance against ACCase- (fenoxaprop-P-ethyl and pinoxaden) and ALS-inhibitors (mesosulfuron & iodosulfuron) as well as for a photosystem II inhibitor (chlorotoluron). For two populations sensitive as well as the most resistant F2-individuals were pooled separately and used for bulked segregant analyses of gene expression by means of a 3'-specific next generation sequencing technique (MACE = massive analysis of 3'-cDNA ends). Gene expression levels were determined by counting the frequencies of MACE reads found for each contig in the different metabolic resistant parental populations, the sensitive wild type and the derived bulks and normalized to "reads per million" for comparability. Candidate genes which exhibited a consistent higher expression level throughout the resistant parents and resistant F2-pools, were verified on the bulked material and tested by means of quantitative PCR in 230 F2 individuals resulting from the six different crosses to correlate the mRNA expressions with the herbicide resistant phenotypes. qPCR expression data were normalized to an optimized subset of proprietary reference genes.

So far eight candidates have been validated which belong to glutathione-S-transferase, glycosyl-transferase, cytochrome P450 monoxygenase gene families and three other - less well annotated - genes which are likely to be involved in stress signaling or detoxification pathways. Different combinations of accumulated gene expression levels are required to separate resistant from sensitive F2 individuals depending on the parental cross from which they originated. This corresponds to our working hypothesis that metabolic resistance is a polygenic trait determined by varying combinations of genes emerging from a larger pool of potential candidates. However correlation of cumulative expression levels and resistance phenotype did not work for all six populations equally well which suggests missing candidate genes yet to be identified from the tested blackgrass material.

## CONYZA SPP.: DISTRIBUTION AND EVOLUTION OF HERBICIDE RESISTANCE

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*Conyza* species are troublesome weeds infesting various habitats in Europe and the Mediterranean region. Until now more than 80 cases of herbicide resistance were reported in *Conyza* species. A survey of *Conyza canadensis* (CC) and *Conyza bonariensis* (CB) populations was conducted across Israel; seeds of more than 90 populations were collected and characterized. Most of them (86%) were found in habitats that had been dramatically impacted by human activities (roadsides and urban habitats) suggesting these species are anthropogenic. The majority of *Conyza* spp. populations (90%) identified as resistant to pyrithiobac-sodium, indicating possible natural resistance to this herbicide. Populations CC21 and CC17 were highly resistant to all tested ALS inhibitors due to a substitution in the ALS gene from Trp<sub>574</sub> to Leu. In other tests they were found to also be triazine-resistant due to a substitution in the *psbA* gene from Ser<sub>264</sub> to Gly. Since glyphosate resistance was reported in locations were some of the *C. bonariensis* populations were collected, additional tests conducted to detect glyphosate resistant (GR) biotypes. Another population that was collected from road sides, AH (CB47), was found to be highly resistant to glyphosate compared to the glyphosate sensitive (GS) CB18 population.

C<sup>14</sup>glyphosate translocation pattern was typical and similar in both AH and CB18 populations but shikimate accumulation was lower in GR comparing to GS. Neither amino-acid substitution nor differences in EPSPS sequence nor its RNA expression level were found between GS and GR. However, transcript expression analysis of three MDR transporters showed a rapid increase 24 HAT of glyphosate in GR compared with GS. These results may indicate that glyphosate uptake and translocation patterns are similar in both GR and GS biotypes but they may differ quantitatively in the herbicide level sequestered away from the chloroplast and transported to the vacuole, hence reducing the level of EPSPS inhibition in the chloroplast.

#### INVESTIGATION ON RESISTANCE OF APERA SPICA VENTI IN LITHUANIA

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Sulfonylurea herbicides are widely used for grass and broadleaf weed control in winter cereals in Lithuania, but low efficacy against *Apera spica venti* sometimes are seen in commercial fields. The goal of investigation was to evaluate presumptive resistance of *A. spica venti* to herbicides in winter wheat in South Lithuania.

Over the period of 2012–2013 twelve winter wheat fields were chosen according to compliance of farmers about low efficacy of herbicides. 60–70 panicles of *A. spica venti* at the ripening stage were taken from each field randomly. Herbicide efficacy was tested in the pot experiments: 16 pots from each sample were sown with *A. spica venti* seeds (172 pots in total). Seed samples were sown in 8 cm Jiffy pots containing a loamy silt soil (LSI; pH: 7.4, sand (%) 19, silt (%) 60, clay (%) 22 and C org (%) 2.2). Pots are watered from below. The greenhouse temperature regime is 22/15°C day/night photoperiod lasted 16 hours.

Different actions of herbicides: ACC inhibitors, ALS inhibitors, photosystem II inhibitors and mitosis inhibitors in recommended rates were spray applied after the *A. spica venti* germination (BBCH 12) using a laboratory track sprayer equipped with a flat-fan nozzle delivering a spray volume of 300 l/ha at 200 kPa. The evaluation was done three weeks after application by visual assessment of the damages (%) in comparison with the untreated control. Classification of susceptibility was done in such way: 100-80% were classified as susceptible 79-50% - intermediate and <50% resistant.

DNA analysis and target site resistance was measured by pyro sequencing method. Pyro sequencing is a DNA sequencing technique based on sequencing-by-synthesis enabling rapid real-time sequence determination. For herbicide metabolism analysis, two tillers of each plant were incubated with radio labeled fenoxaprop-p-ethyl. Extracts of the plant samples were analyzed using HPLC analysis with a Ramona 92 detector. Analyses were made at the laboratory of Bayer CropScience (Germany).

Resistance on sulfonylurea herbicides with active ingredients': iodosulfuron-methyl-sodium; sulfosulfuron; pyroxsulam, florasulam, aminopyralid was found in seven *A. spica venti* populations. DNA sequences analyses of silky bent grass showed, that in the studied populations occurred target-site resistance for ALS inhibitors. However one case of metabolic resistance of *A. spica venti* to ACC inhibitors was detected.

Investigation of DNA sequences of silky bent grass population in two populations showed in accordance 25 % and 43 % of samples has homozygous genotype resistance, when allelic combination includes two dominant herbicide-resistance genes. Hybridize homozygous genotype of silky bent grass germ cells, there would be also homozygous weeds grown from seeds – they would be resistant to ALS groups' herbicides. The history of the field with *A. spica venti* metabolic resistant population showed that fenoxaprop-p-ethyl was frequently used for the weed control in this field. In majority of fields with resistant *A. spica venti* minimal soil tillage was used and growing of winter cereals were repeated several years.

#### A FARMER SURVEY ON HERBICIDE RESISTANCE IN LOOSE SILKY BENT GRASS IN THE CZECH REPUBLIC

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Reports on the results of a farmer survey of the abundance and distribution of the major weed species and weed control methods used in winter crops. A questionnaire was designed with questions focusing not only on potential effects related to herbicide resistance in *Apera spica-venti*, but also on background information about cultivation methods. Farmers were also requested to nominate the most difficult weeds to control in winter cropping systems. Questionnaires were completed by farmers with assistance from agricultural advisors.

Based on the farmers' reports of poor control of *A. spica-venti* by ALS-inhibiting herbicides during the growing season, the fields were selected to collect *A. spica-venti* seeds. The history of the field was obtained from the farmer in order to identify factors that favored the selection of the resistance in the respective field. In the main area of its occupancy, more than 100 populations were analyzed. The frequencies of populations resistant to broadly used ALS inhibitors were assessed by bioassays. The data obtained from survey and testing were saved in the WeedMap software (www.weedmap.eu).

The questionnaire response rate was low and therefore not adequate for statistical evaluation. Given that the survey is complex, covering a large number of questions and issues, the main trends can be observed. The results indicated that farmers found *Apera spica-venti* to be the most widespread and abundant weed in the winter cropping systems. Some weeds, such as *Poa annua*, *Geranium pusillum* and *Bromus* ssp. showed regional propensity, indicating the existence of differences in the importance of the individual species. On contrary, the decrease in area and density was mentioned in *Elytrigia repens*, *Centaurea cyanus*, *Cirsium arvense* and *Capsella bursa-pastoris*, respectively. A large proportion of farmers replied that the weed problem remains unchanged or is worsening (40%) over past 10 years.

It was observed that increase in some species has been increased by adoption of reduced tillage practiced and sulfonylurea herbicides use. Furthermore, the simplification of cropping systems consisting of winter crops exclusively contributed to this increase. Inhibitors of ALS were by far the dominant herbicides used for the control in winter crops. In a few cases, some herbicides had to be excluded from use due to herbicide resistance.

More than 80% of *A. spica-venti* populations tested were found to be resistant to sulfonylureas and triazolopyrimidines. Usage of the herbicides with the same mode of action for a longer period significantly increased the probability of herbicide resistance occurrence. Herbicide resistance affects large numbers of fields, especially in western and southern regions, and is expected to be an increasingly important problem requiring integrated solutions.

This work was financially supported by the National Agency for Agricultural Research, project No. QJ1310128.

## ASSESSMENT OF HERBICIDES RESISTANCES CASES IN FRANCE

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Resistant weeds are an important problem in weed management in France. For the moment, this problem occurs mainly in cereals but is expected in other crops.

In 2010 a national working group was created in the framework of the French Plant Protection Association (AFPP), bringing together technical institutes, pesticides suppliers, research institutes and some advisers. This group made an assessment of resistance cases in France. This update identifies weeds species for which cases of resistance have been found, the range of herbicides to which resistance has evolved and the mechanisms responsible.

At the national level, this update indicated that, for instance, resistance to ALS herbicides have been found in blackgrass (*Alopecurus myosuroides*) rye-grass (*Lolium sp.*), barren brome (*Bromus sterilis*), loose silky-bent (*Apera spica-venti*) poppy (*Papaver rhoeas*) and mayweed (*Matricaria recutita*) populations. Although this information is quite interesting, it is not really useful because it does not help local advisers to have a good idea of the resistance situation in their region.

To address this limitation, it was decided to make assessments at regional level (there are 22 regions in France, gathering together 96 departments). A monitoring, with random analysis, would have given an idea of resistance frequency in every department but it would have called for a lot of resistance tests. Without any means for such a study, it was decided to take an inventory of the resistance cases. This assessment was made by consultation between all local actors: pesticide suppliers, local advisers, technician from technical institutes... Records per species are carried out per departments and reported on a map with different colors according to the frequency of confirmed occurrence: first case, 2-5, 5-20 and more than 20 cases.

For example, this assessment showed that in Côte d'Or department the first case of ALS blackgrass resistance occurred in 2004. There are now many blackgrass resistant populations and it is impossible to count them. All the department is concerned except Morvan area (south west of the department). In Côte d'Or department there are also some cases of *Lolium* resistant populations (less than 20 on the area) and brome resistant populations (less than 5). First cases of poppy resistance have been detected around Dijon city in 2010 and there are less than 5 cases in the area.

This assessment has been made in more than 40 departments in France and is still on going in other regions. This assessment provide local professionals with information about the risk of occurrence of resistant species very locally, which can determine the likelihood of implementing prevention strategies. It is also very interesting at national level, e.g. poppy resistance cases are identified in almost every region, but in every area the number of cases is quite small.

## MONITORING HERBICIDE RESISTANCE IN CHRYSANTHEMUM CORONARIUM INTUNISIA

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In the last decades, herbicides have become the foundation of weed control. However their repetitive use, and the limited number of modes of action available, has led to the development of resistance. In Tunisia, the first report of herbicide treatment failure against the dicotyledonous *Chrysanthemum coronarium* is observed.

The objective of this study was to understand the response of these biotypes to sulfonylureas (SU), triazolopyrimidines (TZP), synthetic auxin herbicides, and mixtures between them, to determine whether there are cases of resistance and which alternative herbicides can solve the problem. A total of five herbicides were tested at the commercial dose (1X), 5 X and 10 X the commercial dose.

A bioassay was conducted in a field where treatment failures were observed. Each treatment had four repetitions and was compared with an untreated control. Efficacy was evaluated by the number of survival plants. The first results showed a reduced efficacy of ALS-inhibiting herbicides. The molecular basis of this resistance will be investigated.

### WHAT IS THE STATUS OF RICE SYSTEMS IN URUGUAY IN TERMS OF HERBICIDE RESISTANCE?

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Rice production systems in Uruguay have been characterized by rotations with pastures, low inputs and very high yields. Nevertheless, high costs and low rice prices have switched systems to more crop-intense rotations, losing the pasture phase in a large area. Such intensification includes more fertilization and herbicides use, between other chemicals. It is well known that increasing the frequency of herbicide use and reducing crop rotation is one of the major causes of evolving herbicide resistant weeds.

Barnyardgrass (*Echinochloa* spp.; ECH) is the major weed in these rice systems, and it has to be heavily controlled in order to get good yields. This weed is usually controlled using various herbicides in sequences -pre and post emergence- and tank mixes, but lately the number of applications and herbicide doses has been raised in order to obtain acceptable control. This situation made us alert due to the risk our ECH would be evolving to resistant biotypes. In the low profitable context mentioned above, dealing with resistant weeds would be highly undesirable.

A previous study carried on with *Echinochloa crus-galli* (L.) Beauv from the east part of the country detected various biotypes resistant to quinclorac; moreover some of them coming from Clearfield systems were also resistant to imidazolinones.

Geo-referenced ECH samples from rice fields within the center and north of Uruguay started to be collected in summer 2013, including spots where farmers suspected of having a resistance issue; apparently the herbicide applications were well done but weeds were not controlled. Following the Herbicide Resistance Action Committee (<u>http://hracglobal.com/</u>) protocols, dose response assays were conducted with most of the herbicides used in post emergence in current Uruguayan rice production, as quinclorac, propanil, penoxsulam, bispyribac and imidazolinones. Various *Echinochloa crus-galli* biotypes showed high resistance to quinclorac, and only a few of them expresses some resistance to ALS inhibitors; fortunately none of them survived when propanil was utilized. Tests with *Echinochloa colona* and E. *crus-galli* var. *mitis* collected from the same fields are still remaining, as well as assays with grass herbicides profoxydim and cyhalofop. At the same time data from field's management history is being collected in order to understand why resistance has or has not evolved.

Despite of efforts invested in training and educating farmers and technicians about the risk of the evolution of resistant types and the importance of prevention as the main factor, resistance has been detected. Nevertheless, these results are not surprising in the intensification production context explained above, where the need of a present economic result is more important than compromising the future sustainability of rice systems. These efforts are the first approach to know about the resistance status in Uruguayan rice; we have to keep on monitoring problematic situations and working on extension to awareness farmers and the industry in order to slow down the evolution of resistant weeds.

### A MODELLING TOOL FOR ASSESSING HERBICIDE RESISTANCE DEVELOPMENT IN ARABLE WEED SEEDBANKS

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The recent loss of herbicide active substances, coupled with a lack of development of new modes of action is leading to an over reliance on a limited number of existing products, many belonging to high risk resistance groups. This continues to aid the development and spread of herbicide resistance. Current experimental work in the UK is investigating the risk of ALS-resistance in broad-leaved weeds and attempting to develop potential solutions to prevent a wide scale increase in ALS resistance, with particular emphasis on common poppy. However, the long-term impacts of potential control measures on the resistance profile of the weed population in the seed bank are not being investigated.

The Chemical Regulations Directorate in the UK, have funded the development of a tool to assess the ability of different combinations of cultural practices and herbicide regimes to provide management of the resistance status of the weed seed bank and maintain adequate levels of control throughout a crop rotation. This tool will be used to assess the potential impacts of novel herbicides on resistance as part of the regulatory framework. It is not aimed therefore at providing a detailed simulation of weed dynamics, but in providing a suitable reproduction of weed dynamics to ensure that the timing of cultivation and herbicide application events with respect to weed and seed bank dynamics are captured, as it is this timing that drives the development of resistance within the seed bank.

The tool has initially been developed for common poppy and the underlying model of the weed and seedbank dynamics incorporates the impact of cultivations on movement of seeds between deep and shallow layers in soil; seed survival in soil (accounting for viability, dormancy status and predation); temperature and rainfall threshold effects on weed emergence and temperature and rainfall driven growth, development, survival and seed production. A pragmatic approach to the modelling of the weed dynamics has been taken, integrating empirical approaches with simulation modelling. For example, timing of emergence is fixed, following a normal distribution, with temperature and soil moisture thresholds used on a daily basis to determine daily emergence, whilst the growth of the weed after emergence uses a temperature and rainfall driven simulation model.

The tool allows the user to define scenarios for the management of the crop (timing of operations and number of herbicides to be applied) as well as selecting different climate scenarios, inheritance mechanisms and levels of cross pollination. Initial scenario assessments using the tool have shown that timing of herbicide applications relative to the age structure of the weed population has a significant impact on the development of resistance. We aim to address this by creating a method to apply herbicides at optimal timings based on the mix of growth stages of the weed population and time since last herbicide application.

#### SIMULATION EXERCISES ON LONG-TERM MANAGEMENT OF WIDESPREAD HERBICIDE RESISTANCE IN A FIELD WEED POPULATION

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Herbicide resistance is a growing threat to arable land use in many parts of the world. Meanwhile resistance occurs in various weed species and is not restricted to single patches, but can effect production decisions for whole fields. Population studies of resistance on a field level are scarce. The development of herbicide resistance on population levels occupying whole fields is an appropriate exercise for population modeling and simulations.

For this purpose we developed a population dynamic model, which takes into account the genetics of herbicide resistance. The model PROSPER combines the population dynamic approach of our working group in Rostock and the genetics proposed by a model developed in Australia, called PERTH. The first is a model where the parameters are statistically estimated for the whole population. The second is an individual based dynamic model which estimates a (metabolic) resistance level for every plant based on a specific genetic situation. The combination of both allows estimating the gene flow in a weed population while counting for the stochastic nature of resistance evolution. Population parameters flow into PROSPER from an external source.

For the simulation exercises we selected *Galium aparine* as example weed species. Although this species is so far hardly affected by herbicide resistance, the patchy occurrence of the large-seeded species which result in highly variable parameters on a field population level encouraged us to use it as model weed. Stochastic population parameters were calculated from data of a long term experiment. Parameters are in the majority calculated using mixed effect models. In the genetic part we assume a simple situation: 4 resistance genes are working, epistasis is ignored and co-dominance for all genes is assumed..

We created 16 scenarios: 4 different initial resistance levels in the field and 4 different management strategies including sowing of susceptible weed seeds. Simulations show potential "points of no return" in weed resistance levels. They further estimate the potential timespan left for or required to restart arable land use on the field when relying on seed production of the local resistant plants only.

The population model PROSPER is drafted in R and will be published as package. Within this public software shell, model application with various sources of data will be possible.

## WEED PAM: A RAPID IN-SEASON HERBICIDE RESISTANCE DETECTOR

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The management of *Alopecurus myosuroides* (blackgrass) in Europe is being more complicated due to the herbicide resistance. Field and greenhouse experiments were conducted to calibrate a new in season weed resistance detection to herbicides shortly after application. A greenhouse bioassay and two field experiments were carried out in southwestern Germany with *Alopecurus myosuroides* in winter wheat. Herbicides in different mode of actions were tested. Weed response to herbicides was measured with a chlorophyll fluorescence imaging sensor.

The maximal PS II quantum yield significantly decreased in sensitive Alopecurus myosuroides population after 3 days after treatment. The data corresponded to the greenhouse bioassay for herbicide efficacy. Molecular analysis for target site resistance (TSR) was carried out afterwards in lab with resistant plants. The new technology tested was capable for quick classification of sensitive and resistant Alopecurus myosuroides populations. It can be used for optimizing management of strategies to control herbicide resistant weeds.

Keywords: *Alopecurus myosuroides* (blackgrass), herbicide resistance, quick sensor detection, chlorophyll florescence.

#### FAST DISCOVERY OF SSR MARKERS IN ECHINOCHLOA CRUS-GALLI USING NEXT GENERATION SEQUENCING TECHNOLOGY

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Simple sequence repeats (SSR), a special motif of repetitive DNA, have been utilized for genetic analysis such as genetic diversity and population structure. However, development of SSR has been traditionally difficult, labor consuming, and expensive. Next generation sequencing (NGS) technology makes the efficient and cheap identification of numbers of SSR in plant genomes possible. The objective of this study is the development of SSR marker in *Echinochloa crus-galli* using NGS technology.

We adopted Illumina sequencing for the discovery of SSR and obtained 34 Gbp of nucleotide information from 337,650,586 sequence reads. A total of 44,257 di-/tri-nucleotide repeat motifs were identified; di-repeat motifs were 2.89-fold more common than tri-repeat motifs. [AT]<sub>n</sub> repeat were the most frequent in di-repeat motifs and [GCG]<sub>n</sub> repeat were the most frequent in tri-repeat motifs. We screened 153 primer pairs, which have 75-400bp amplified product and more than 10 repeat motifs, from 12,270 tri-nucleotide repeats and 31,987 di-nucleotide repeats. We examined PCR amplification success using five fenoxaprop-p-ethyl resistant *Echinochloa crus-galli* and three susceptible *E. crus-galli* accessions. Finally, 32 SSR polymorphic markers were chosen. The number of alleles was four to 11. The SSR markers developed here will be used for understanding the genetic structure, evolution, gene flow of the genus *Echinochloa*.

## MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF WEEDY SPECIES OF THE GENUS *CHLORIS* SPP. FROM CUBA

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The total cultivated area of citrus in Cuba is around 40 000 ha and citrus fields are distributed throughout the island. In 2013, a weed survey was conducted focusing on grass weeds, which were increasingly difficult to be controlled with glyphosate in those fields in Cuba. Of these species, three species of the genus *Chloris* were chosen to present the highest density in citrus fields that had been historically treated with glyphosate. The main objectives of this work were to quantify the resistance in *Chloris* treated and untreated populations with glyphosate, and to carry out the characterization of these species by measuring morphological parameters and using molecular markers.

To determine the efficacy of glyphosate on the species effective mean dose (ED<sub>50</sub>) parameter was studied. The results showed different values of ED<sub>50</sub> and ranged from 151 to 823 g ea ha<sup>-1</sup>. The resistance factors (RF) ranged from 1.7 and 3.3 between species of *Chloris* treated and untreated populations with glyphosate. The results showed that the order of efficacy of glyphosate was *C. ciliata* Sw. (RF=3.3) > *C. inflata* Link. (RF=1.7) > *C. elata* Std (RF=2.4). Morphological characterization showed significant characters in each species of which can stand out mainly the fertile floret of *C. inflata* is glabrous; *C. ciliata* has the awn of fertile floret smallest; and *C. elata*, besides having the leaves on average twice as width as *C. ciliata* and *C. inflata*, *al*so has the largest number of spikelets per raceme and per inflorescence indicating that this species is capable of producing more seeds than the other two species.

The resolution of *Chloris* species at the molecular level, based on AFLP analyses, was fairly consistent with morphological analysis results. The individuals analized were separated clearly in three groups. These results showed different levels glyphosate resistance in the three species studied and their respective populations. Future studies should be conducted about the mechanisms to corroborate the glyphosate resistance in these *Chloris* species.

Keywords: glyphosate, *Chloris* spp., ED<sub>50</sub>, morphological parameters, botanical characterization.

## **SESSION IIIB**

## WEED ECOLOGY

#### UNDERSTANDING SCALE-DEPENDENT RELATIONSHIPS BETWEEN SPATIALLY HETEROGENEOUS POPULATIONS OF ALOPECURUS MYOSUROIDES AND SOIL PROPERTIES

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Weed populations are often heterogeneous within the environment, showing aggregation in patches with varying size, shape and density. Although there tend not to be consistently exhibited patterns across species, the distribution of weeds can be strongly affected by many abiotic factors including soil type, pH and moisture. An understanding of these relationships can allow the use of precision management techniques through the development of models to predict the location of weed patches.

The spatial variation of an environmental property, such as the phosphorus concentration in the soil, can result from multiple and interacting processes operating at several spatial scales and, as a consequence, the variation observed typically changes across spatial scales. This means that relationships between two variables found at the one scale might not hold at another. Nested sampling designs allow the study of correlations between populations and environmental properties at multiple spatial scales. We used a nested sampling scheme with 136 sampling points to quantify the scale-dependent relationship between patches of *Alopecurus myosuroides* Huds. and soil properties in two fields of winter wheat.

Often weak marginal correlations were found between *A. myosuroides* and soil properties, such as soil moisture content, organic matter and clay content, across the whole data set. These masked stronger correlations at coarse scales (>50 m). The correlations between black grass counts and the properties soil moisture and phosphorus concentration by Olsen P also changed sign with scale, indicating not only that the strength of relationship was related to the scale at which it was observed but also the direction.

Ongoing glasshouse trials will allow the study of the effect of soil organic matter and water on various aspects of the *A. myosuroides* life-cycle, including germination and seed return, in a more controlled manner. The results from this work will allow parameterization of a spatially explicit life-cycle model for *A. myosuroides*, which will be parameterized according to the corresponding relationships with soil properties.

#### DOES PLANT ACTIVATOR APPLICATION HELP THE ANTIOXIDANT DEFENSE OF TOMATO PLANTS DURING BROOMRAPE INFECTION?

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Broomrapes (*Orobanche* sp., *Phelipanche* sp.) are invasive weeds that damage major agricultural plants such as tomato, sunflower, lentil, etc. Broomrape seeds germinate after the release of strigolactone from host plants' roots. In this study, we focused on the antioxidant enzyme levels of root and leaf tissues of tomato (cv. Rio Grande) and ISR 2000<sup>™</sup> (Induced Systemic Resistance) application during broomrape infection. ISR 2000<sup>™</sup> is a plant activator and product from extract of cell wall material of yeast that is widely used in agricultural production.

Tomato seedlings were grown under controlled conditions (24±2 °C, 16/8 hrs light/dark, 55-65% RH) in a hydroponic culture with Hoagland solution, and experimental design was included three independent replicates. Experiment groups were; (A) Control (tomato plants without broomrape or ISR 2000), (B) Broomrape infection (tomato plants infected with broomrape), (C) ISR 2000 treatment (tomato plants treated only with ISR 2000) and (D) ISR 2000 and broomrape (tomato plants infected with broomrape and treated with ISR 2000). Before application, *Phelipanche aegyptiaca* Pers. seeds were induced to germinate with GR-24. Seedlings (30 d) were treated with ISR 2000 via recommended dosage (100 ml/da) on the first day of following the infection. Although broomrapes are parasitized to the root tissues of host plants. Root and leaf tissue samples were analyzed for antioxidant enzyme activities (SOD, POX, APX, GR, CAT) and chlorophyll (Chl a, Chl b) and carotenoid pigments amounts on the 0<sup>th</sup>, 3<sup>rd</sup> and 7<sup>th</sup> day after treatment. Statistical analyses were done with one-way ANOVA and Tukey HSD test via SPSS (20.0) program.

Compared to control plants, total chlorophyll amount (mg/g FW) of plants in the ISR 2000 and broomrape treatment was increased by 40%. SOD, POX, APX, GR and CAT activities were approximately 3–8 fold higher in root tissues. Otherwise, SOD, POX, APX and CAT activities were also increased 2-4 fold compared to control plants, whereas GR activity was decreased in the leaf tissues in the end of experiment.

GR and APX activities were increased 3 and 2 fold respectively with ISR 2000 treatment during broomrape infection compared to non-infected plants. It was determined that in the root tissue of tomato plants, the antioxidant defense system was induced with ISR 2000 treatment during broomrape infection. Moreover, our physiological analyses indicated that this induction was achieved via the ascorbate-glutathione pathway in root tissues of tomato plants. The plant activator was more effective in root tissue than in leaf tissue. According our results it can be concluded that the ISR 2000 treatment can provided better protection during broomrape infection compared to untreated tomato plants.

Keywords: Broomrape; Tomato; Antioxidant Enzymes; ISR 2000.

#### IMPORTANCE OF HABITAT, LOCAL MANAGEMENT AND LANDSCAPE COMPOSITION ON THE ABUNDANCE OF WEED SEED-EATING CARABID SPECIES IN CROP FIELDS

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Weed seed predation by carabids is a naturally occurring ecological function that could substitute for chemical weed control. Although variable amongst species, the daily consumption of weed seeds by carabids can be very important under laboratory conditions. In addition, large-scale studies indicate that in-field abundances of seed-eating carabids are correlated positively with the level of weed seed predation and negatively with the rate of increase of the weed seed-bank.

The composition of the carabid community of arable crops is known to be affected by the type of habitat, *e.g.* crop type and the local agricultural management. The landscape context of arable fields can also affect the abundance of carabid species in a focal field. For example, some results suggest that the abundance of strictly granivorous species may depend on the occurrence of particular habitat types in the vicinity, such as grassland and oilseed rape that subsidize their phytophagous diet. Here, we analyzed the local habitat preferences and the effect of the landscape context of arable fields at different spatial scales on the abundance of seed-eating carabids, with focus on the species that have been shown to consume particularly high levels of weed seeds.

Using data collected in 164 arable fields of four crop types across the UK (FSE database), the variation in abundance of 9 seed-feeding carabid species was analyzed with generalized linear mixed models. Explanatory variables were: (i) crop type and management intensity (Field management); (ii) occurrence of oilseed rape and grassland adjacent to the field (Neighbourhood); and, (iii) proportion cover of oilseed rape and grassland within a 5km<sup>2</sup> grid squares (Landscape). Our analyses suggest that crop type has a major impact. Species belonging to the genus *Amara* were found preferentially in oilseed rape. Thus, we considered these as specialist granivores. Conversely, granivores of the *Harpalus* genus were more generalist in terms of habitat choice. The intensity of field management had a negative effect on the abundance of several species, irrespective of their trophic guild. We detected an effect of the presence of oilseed rape and grasslands on the abundance of carabids at different spatial scales (Neighbourhood/Landscape). The occurrence of grasslands in the neighborhood of our focal arable crops increased the abundance of granivores in spring. At a larger spatial scale, the landscape context of our field had a strong effect on all seed-feeding species, especially for generalist omnivores.

Our results indicate that the abundance of seed-eating carabids in arable fields is driven by factors acting at local, neighboring and landscape scales. This would suggest that there is a diversity of managements that could be used to enhance weed seed predation in arable fields. Further investigations are required to: (i) refine the contribution of individual carabid species to weed seed predation; and (ii) understand the population dynamics of seed-eating carabids in agricultural landscapes.

#### DENSITY-DEPENDENT SEED PREDATION IN MAIZE MONOCULTURE

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Post-dispersal seed predation is the consumption of mature seeds on the soil surface. Seed predation can prevent spatial weed spread if seed predators consume more seeds in areas with high seed density than in areas with low seed density (direct density-dependent). In this study, the aim was to estimate the response of seed predators to increasing seed densities of *Echinochloa crus-galli* (L.) P. Beauv. in maize. Depending on the main type of predator, vertebrate or invertebrate, the response can be directly or inversely density-dependent. Vertebrate seed predators, such as granivorous rodents, are more mobile than invertebrate seed predators, such as granivorous carabid beetles, and are, therefore, more likely to find and exploit patches with high seed densities. As in our area, we probably have a combination of different types of seed predators, the exact response cannot be predicted.

In 2014, an experiment in two maize fields was conducted in North-Eastern Germany to examine the response of seed predators to different densities of weed seeds of *E. crus-galli*. Thirty patches of 1.5 m × 1.5 m were established per field, and seeded in August 2014 with either approx. 1200, 2400, 4800, 9600 m<sup>-2</sup> or no seeds (six replications per density per field). Prior to seeding, each patch was equipped with a 0.1 m<sup>2</sup> seed tray, filled with seed-free soil. These seed frames were seeded with the same seed density as the surrounding plot, but the number of seeds was determined much more accurately. Seed trays were removed one day prior to maize harvest in October 2014. Seeds were separated from the soil in the seed trays *via* elutriation, followed by a flotation technique with NaCl, and counted manually.

The relationship between the initial number of seeds and the number of seeds retrieved after exposure will be determined and used to estimate the degree of density-dependence. Results on the relationship between the seed density and predation rate will be presented and discussed.

We thank the DFG for funding of the project (WE 5040/2-1) "Testing the weed control potential of seed predators in agroecosystems" and farmers that host our experiment for their kind support and hospitality.

#### CROP COMPETITIVE INTERACTIONS DRIVE THE FUNCTIONAL STRUCTURE OF WEED COMMUNITIES WITHIN ARABLE FIELDS

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In arable fields, the crop represents a dominant competitor, due to an early establishment and a high-density sowing, both conferring crop individuals a long-term preferential access to light. While weed-crop bispecific competition is currently well-known for several weed species among the most problematic, no studies have focused on the effect of crop competition on weed communities under real field conditions using a trait-based approach. In this context, we hypothesized that: (i) a functional similarity between crop and weeds plants promotes the performance of the latter, resulting in a trait-convergence pattern at the community scale, suggesting a unique strategy for an optimal capture of resources and (ii) a functional differentiation, by promoting a diversity of strategies of resource capture, allows the coexistence of weed species with dissimilar trait values compared to the crop, while others could be more similar, resulting in a trait-divergence pattern.

We set up a semi-experimental design in five winter wheat fields located in the LTER Zone Atelier Plaine & Val de Sèvre (central western France) in 2013. We manipulated the presence and absence of nitrogen fertilization and crop in a cross-factorial design in unsprayed herbicide areas. We measured four functional traits related to weed response to light and nitrogen availability: canopy height, specific leaf area, leaf mass fraction and shoot nitrogen concentration, as well as the above-ground biomass, considered a surrogate of the plant performance. Up to 20 weeds and five crop plants were randomly selected per treatment per field in early June 2013 at the beginning of winter wheat flowering, over a period of two weeks. We used generalized linear mixed models to analyse the influence of the crop presence and nitrogen fertilization on weed trait values and individual above-ground biomass production.

In presence of the crop, we highlighted that a functional similarity allows the production of a higher individual above-ground biomass of weed plants. This trait-convergence pattern, observed for all traits, is also supported at community-scale. This pattern seems mainly due to a great and early crop canopy closure which reduces light availability, thus promoting a shade avoidance syndrome characterized by stem elongation and a larger investment in structural tissues to overtop the crop canopy. Although already known for a few species, our results generalize this trend at the community scale for a greater number of weeds. By contrast, in non-sown treatments, the community predominantly showed a random functional structure, and sometimes a trait-divergence pattern. This result suggests either resource partitioning, or the coexistence of erect and prostrate growth strategies, which are both able to produce high quantities of individual above-ground biomass.

#### ATTEMPTING TO SEPARATE RESOURCE COMPETITION FROM ALLELOPATHIC ROOT INTERACTIONS

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We are working on a method to study allelopathic effects of below-ground root interactions between two plant species. The objective is to separate resource competition for water, nutrients and light from phytotoxic root interactions between an allelopathic donor plant (cover crop) and a sensitive receiver plant (annual weed).

In a first step, we carried out experiments in non-sterile soil in pots under controlled conditions in a phytotron. Water and nutrient supply were kept constant in sufficient amounts throughout the experiment. The effect of shading was evaluated by the presence or absence of vertical nets between the competing plants. Growth-repressing allelopathic root interactions were studied by separating the rhizospheres of the weed and the cover crop species with impenetrable plastic barriers underwent one set of conditions and by allowing interplant root contacts under another. The growth-repressing effect of *Fagopyrum esculentum* (common buckwheat) and *Sorghum bicolorxSorghum sudanense* (sorghum sudangrass hybrid) on *Amaranthus retroflexus* (L.) (redroot pigweed) was very strong (91% and 68%, respectively). It was due both to competitive shading effects (64% and 21%, respectively) and root interactions (75% and 60%, respectively) of potentially allelopathic nature.

In a second step, we wanted to evaluate the growth-repressive effect of shading of several cover crops in a field trial. In 2013, three cover crops (*Fagopyrum esculentum, Sorghum bicolorxSorghum sudanense* and *Brassica juncea*) were tested, whereas in 2014, 13 different cover crops were studied. Results from the two field trials were very different due to variable weather conditions in the two years. In 2013, shading was predominant as a weed-suppressive factor. In 2014, redroot pigweed growth-suppression by shading was absent due to slow development of the cover crops. However, partial weed suppression was observed.

#### EFFECT OF HERBICIDE TREATMENTS ON THE ASSEMBLY OF WEED COMMUNITIES

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Understanding the response of weed community assembly to herbicides is important for designing sustainable weed management strategies. The use of functional traits has been instrumental in relating the response of weed species to human and environmental selection pressure. However, due to the complex interactions among management practices, further advances now require an experimental approach to isolate the effect of targeted practices. Studies dealing with herbicides have been mainly oriented towards the level of control of particular weed species. Few studies have examined the effect of herbicide treatments on weed species diversity and composition although herbicides are still the main way to control weeds. Therefore, herbicides are expected to be the main driver of the flora composition. In this study, we tried to improve our understanding of weed community assembly rules and the role of herbicides, using a sequential sampling scheme for the weed flora.

A large-scale weed survey, including 1440 arable fields across France surveyed between 2002 and 2010, was used. In each field, weed surveys were performed in one 2000 m<sup>2</sup> quadrats in autumn and spring, in areas treated with and without herbicide. We focused on weed communities in oilseed rape (OSR) and winter wheat (WW). Diversity indices (species richness, abundance and proportion of common and rare species) were analyzed in relationships to management practices, including the Treatment Frequency Index (TFI; the number of full doses applied). We computed several indices to quantify the changes in the density of species (CDS) between control and treated plots (CDS<sub>treatment</sub>) or between the two sampling dates (CDS<sub>season</sub>). The CDS indices were then used as response variables in regression trees to identify the relationship with species traits. Twelve traits were used, mainly related to phenology, competition or regeneration strategies, but also the degree of herbicide tolerance.

Species richness and abundance were not correlated to the TFI. In OSR, the TFI was negatively correlated to the mean decrease in abundance. In WW, TFI was positively correlated to the proportion of common species and negatively correlated to rare species. By grouping species based on their ability to maintain high densities after herbicide treatment, the importance of traits related to phenology (germination start, flowering duration) and sensitivity to herbicides became apparent. For example, in oilseed rape, species belonging to the Brassicaceae maintained higher densities than did Poaceae species.

This study showed that an approach combining multiple scales, i.e. among-fields variations (related to TFI) and within-fields variations (comparing treated versus control plots) can highlight patterns of weed assembly related to the herbicide filter that would not have been detected with simple surveys without semi-experimental data including control plots and several census during one growing season. In the framework of herbicides post-registration survey, the CDS indices can help identifying weeds that persist best after treatments and provide an early detection of a possible decline in control over the medium term. From a more basic point of view, the further development of our approach could improve the understanding of the filtering effect of herbicides treatment on weed assembly.

#### DENSITY-DEPENDENT EFFECTS DURING RE-ESTABLISHMENT OF RARE ARABLE PLANTS

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During the past decades, agro-biodiversity has markedly declined and some plant species are close to extinction in large parts of Europe. Organic farming offers suitable conditions for the conservation of rare arable plants, due to reduced nitrogen input and waiving of synthetic pesticides. However, as these species are almost absent they need to be re-introduced. This study investigates optimal sowing densities of three endangered species, *Legousia speculum-veneris* (L.) Chaix, *Consolida regalis* Gray, and *Lithospermum arvense* L. in terms of establishment success and effects on crop yield.

A field experiment with a partial additive design was performed in the Munich gravel plain (Germany). Winter rye was sown on 40 plots (6.0 m x 1.1 m) at a fixed rate of 350 grains m<sup>-2</sup>. The target species were added with increasing sowing densities (5, 10, 25, 50, 100, 200, 500, 1,000, 5,000 and 10,000 seeds m<sup>-2</sup>) in 30 plots with pure sowing and in ten plots with mixed sowing. Sampling was carried out on eight subplots (0.25 m<sup>2</sup>) per plot, with or without spontaneous weeds. Performance of the disseminated target species was assessed by establishment, biomass, flower density, seed production and germination; crop response was determined as grain yield, thousand grain weight and ear density. Opportunity costs of different sowing methods were calculated in cost-benefit analyses based on marginal costs.

While the target species established and reproduced at all sowing densities, optimal performance was found at 50–1000 seeds m<sup>-2</sup>. Densities <25 seeds m<sup>-2</sup> led to poor seedling establishment, and >1000 seeds m<sup>-2</sup> caused negative density effects. The impact on crop yield followed sigmoidal or hyperbolic functions. Depending on the species, yield losses occurred at >100–1000 seeds m<sup>-2</sup>. Based on these results, we recommend densities of 100 seeds m<sup>-2</sup> for *C. regalis* and *L. arvense*, and 50 seeds m<sup>-2</sup> for *L. speculum-veneris* for successful re-introduction. This would cause management costs of <1000  $\in$  ha<sup>-1</sup>, and crop yield losses <7%.

The study shows that the re-introduction of rare arable plants by sowing in organic fields is a feasible method of restoring agro-biodiversity. Ecological mechanisms that control the effects of sowing density are discussed.

#### WEED SURVEY OF SOYBEAN FIELDS IN NORTH-WEST HUNGARY

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Hungarian soybean (*Glycine soja*/L./Siebold et Zucc.) production is increasing, and it has been planted on over 40,000 hectares annually in recent years. High weed density in a soybean crop usually causes significant yield losses, and its weed control system demands a high level of technological knowledge from farmers.

Fifty-four soybean fields were surveyed across north-west Hungary at the seasonal peak of summer annual weed vegetation in August 2013. Weed vegetation of the fields was sampled in four randomly located 50 m<sup>2</sup> plots. Percentage ground cover of plant species in each plot was estimated visually. Weed species were ranked according to their mean cover values.

Altogether, 83 weed species were recorded. The most abundant species (mean cover value) were: *Ambrosia artemisiifolia* L. (2.16%), *Chenopodium album* L. (1.58%), *Cirsium arvense* /L./Scop. (1.08%), *Convolvulus arvensis* L. (0.85%), *Echinochloa crus-galli* /L./ P.B. (0.75%), *Panicum miliaceum* L. (0.7%), *Setaria pumila*/Poir./Schult. (0.38%), *Elymus repens* /L./Gould (0.32%), as well as *Brassica oleracea* L. (0.23%) and *Helianthus annuus* L. (0.19%) volunteer crops. The most dominant plant families and their proportions of the total weed cover were: Asteraceae (35%), Poaceae (22%) and Chenopodiaceae (18%). On the basis of mean cover value, a pronounced dominance of summer annuals (70%) and geophytes (27%) was found.

This work was supported by project OTKA K111921.

## PARAMETERS AFFECTING PHYTOSOCIOLOGICAL PROFILE OF VINEYARD FLORA IN SWITZERLAND

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Why can we find some weeds in a vineyard and not others? And by which means can we control the evolution of flora in a desired direction? It is complex to answer these questions and, in practice, the management of vineyard flora is often empiric and even sometimes dogmatic.

In order to get a clearer picture, we made a descriptive analysis of the flora of several vineyards so as to highlight phytosociological profiles and their links to human and environmental factors. The ground maintenance practices in these vineyards were diverse (chemical and mechanical weeding, mowing, mulching roller).

We made 436 botanical surveys (species presence and abundance-dominance) on 105 plots spread on the Lake Geneva Region (Vaud, Switzerland) in 2013 and 2014. Viticultural practices were listed by interviewing the wine-growers. Correspondence analysis were carried out in order to assess the linkages between botanical indicators and attributes (Landolt et al., 2010) and cultural practices, soils and climate.

It appears that the level of disturbance allows to draw a clear distinction between the ground maintenance practices. On one hand, chemical and mechanical weeding (high disturbance) tend towards maintaining alliances from the *Chenopodietea* class. One of the most common is the *Fumario-Euphorbion*, consisting of ruderal to competitive ruderals strategists, mainly therophytes and geophytes. On the other hand, mowing and mulching roll (lesser disturbance) tend towards favouring the establishment of a grassland, for instance the *Arrhenatherion*, whose plants show primarily a competitive life strategy and are usually hemicryptophytes. Herbicide use can cause plant selection because of tolerance or inappropriate application. The frequency of mowing has also a major impact on the synusia: frequent mowing promotes hemicryptophytes at the expense of therophytes and selects creeping stem plants. Pedoclimatic factors contribute slightly to explain dissimilarities between samples, despite great differences between the plots.

In conclusion, phytosociological profile of vineyard flora appears to be mainly linked to human actions and their intensity. According to our study, this profile can be relatively predictable at a local scale. This can facilitate cover crop integrated management in viticulture.

In prospect, functional traits characterization linked to the different phytosociological profiles could allow further practical applications. Competition for resources of cover crops in vineyard with low water reserve could be assessed. Ground maintenance practices could then be suggested in order to lead the evolution of flora in a chosen direction.

## PHYTOSOCIOLOGICAL GROUPS OF WEEDS\_OF CROPS IN ORAN, ALGERIA

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We conducted a study on cultigens groups in the region of Oran, Algeria, using surveys of 50 fields, conducted in different crops. These surveys were distributed throughout the study area, taking into account the variability of ecological and agronomic factors.

Eithy species were identified, representing 65 genera and 26 botanical families. The four most prevalent ones were Asteraceae (20%), Poaceae (14%), Brassicaceae (13%) and Fabaceae (9%). Dicotyledons were the most dominant, with 65 species (81%), while monocotyledons were represented with 13 species (18%). Most species were therophytes, present with 46 species (57%), geophytes present with 21 species (26%), or hemicryptophytes present with 13 species (16%).

On the syntaxonomical plan, five distinct groups were identified, namely those associated with:

- Chenopodium album L. and Beta vulgaris Döll in olive,
- Cichorium intybus L. and Daucus carota L. in vineyards,
- Papaver rhoeas L. and Rhaphanus raphanistrum L. in cereals,
- Cynodon dactylon (L.) Pers. and Hordeum murinum L. in citrus,
- Echium plantagineum L. and Ridolfia segetum Moris in fruit orchards.

Groups were attached to different phytosociological classes. Most belong to the class of *Stellarietea mediae*, which is characterized by *Stellaria media* (L.) Vill.. A less frequent class in the region is the grouping of *C. intybus* and *D. carota*, found in the vineyards, which belonged to the *Artemisietea vulgaris*, characterized by *Artemisia vulgaris* L..

The results can help to design a suitable and efficient strategy to control these weeds. Nevertheless, such a strategy cannot be implemented universally, but must be adapted for each particular case. At the end of this study, the control program to be adopted against the weed flora will have a positive impact on qualitative and quantitative aspects of production and on crop yields.

Keywords: phytosociological groups, surveys, syntaxonomical, Oran.

#### NATIONAL SURVEYS OF WEEDS IN ARABLE FIELDS IN LATVIA

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National weed surveys provide valuable information on the composition of weeds in arable fields under intensive farming systems. Since 2000, cereal growing on some farms has become even more intensive and continuous cropping with cereals is now quite common in Latvia.

Several weed surveys have been carried out during the last 60 years, with the first led by botanist Alfreds Rasins in 1947. Two major changes have occurred in Latvian agriculture. In the 1950s, small farms were re-organized into large collective units; the introduction of tractors and herbicides then changed management of traditional cultivation and cropping practice. In the 1990s, opposite changes in agriculture occurred: following the break-up of large collective farms, the existing agriculture system experienced disorganization – the cost of energy and plant protection products increased, with the result that most farmers used herbicides in only few crop fields.

In previous weed surveys, weed species were recorded at 100 points within each observation field, using a 200 cm<sup>2</sup> rectangular frame. Weed densities (plants m<sup>-2</sup>) were calculated from these frequency data. The same methodology of weed assessment was used in weed surveys during the 1980s, during 1994-1996, and in the most recent survey in the project "Integrated pest management for weed control in arable crops for sustainable use of the environment and resources" in 2013-2014 supported by the European Agricultural Fund for Rural Development. The similar research methodology allows us to compare the results obtained from the various surveys. The aim of the project is to develop recommendations for farmers in weed control according to the integrated plant protection systems for field crops, including avoidance of the development of herbicide resistance.

Up to 1947, the most common species in Latvia were *Viola arvensis* Murray, *Cirsium arvense* (L.) Scop., *Achillea millefolium* L. and *Bromus secalinus* L. In the 1980s, *Viola arvensis* was again prevalent, but *B. secalinus* was rare. The results in the 1990s showed that the infestation levels of weeds were high and the number of fields with *Elymus repens* L. as the most abundant species had increased. The results from the most recent project showed that *V. arvensis* and *E. repens* are again the most common species in cereal fields. The abundance of *Equisetum arvense* L. has increased in the last 20 years, which could be the result of continuous cereal rotations and minimum soil tillage. Based on the results of the most recent weed survey, plants of *Bromus* spp., which can be introduced *via* contaminated seed material, were recorded in several fields. Uncontrolled spread of these weeds can cause serious problems for farmers in the future because the control methods for these species are limited in cereals. Other noxious grass-weed species *Avena fatua* L. and *Apera spica-venti* (L.) P. Beauv. have also become more widespread.

## EFFECT OF WILD OAT (AVENA FATUA L.) DENSITY ON DEVELOPMENT AND YIELD OF SPRING WHEAT

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Wild oat (*Avena fatua* L.) is one of the most widespread and feared annual grass weeds in cereals in Latvia, because of its ability to cause significant yield losses. Therefore, the investigation of competition between wild oat and spring wheat is one of the main tasks of the project "Integrated pest management for weed control in arable crops for sustainable use of the environment and resources" supported by EAFRD.

A two-year field experiment was carried out in 2013 and 2014 to evaluate the effects of different wild oat densities on spring wheat cv. 'Zebra' plant development, yield and yield components. The effect of ten different *A. fatua* densities (0, 1, 2, 4, 8, 16, 32, 50-100, 150-200 and 500 plants per  $m^2$ ) was investigated. The experiment was conducted in randomized block design with three replicates and plot size of 3  $m^2$ . Seeds of *A. fatua* were broadcasted on the soil surface immediately after sowing of spring wheat in 2013, but seed germination rate was low. To improve seed germination, *A. fatua* seeds were sown at 3.5 - 5.0 cm depth in 2014. Plant protection measures and fertilizers were applied to the whole trial as necessary according to good agricultural practices. Germination patterns of spring wheat and *A. fatua* seeds were recorded. The total number of *A. fatua* plants that emerged within each plot was determined by counting the number of seedlings per 1  $m^2$  emerging at 5-11 day intervals from crop appearance until the flag leaf emergence stage. Ten plants of both species were marked with a plastic tag within each plot and monitored at regular intervals during the vegetation season. Above-ground biomass, dry mass and nutrient content of plants were determined twice for both species, namely at the crop flag leaf emergence stage and at the crop maturity stage.

Data from the first year of the study showed that spring wheat was more competitive against wild oat, as expected. The sowing method of *A. fatua* seeds used in 2014 improved overall seed germination. Seedling emergence of *A. fatua* started 9 to 13 days after sowing in 2013 and 2014, respectively. The majority of *A. fatua* plants emerged within a 2 - 4 week period after sowing. The grain yield of spring wheat was significantly reduced by higher *A. fatua* densities  $(50 - 500 \text{ plants per m}^2)$  in 2014. There was a tendency towards higher nitrogen content in leaves of *A. fatua* plants, which was effectively used to increase plant biomass and to improve its competitiveness with the crop.

Further field studies are necessary to investigate the impact of *A. fatua* on growth and development of spring wheat, and to confirm spring wheat yield loss especially at low *A. fatua* densities. For agronomy practice, these results can be used to develop decision support system for long-term management of *A. fatua* in spring cereals.

#### **RELATIONSHIP BETWEEN PRECIPITATION AND WEED INFESTATION IN CEREALS**

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Of all meteorological parameters, precipitation is one of the determining factors of weed infestation intensity. Soil tillage significantly influences soil properties and changes the impact of rainfall. They all act on weed seeds in the soil and determine the degree of germination. Finding such relationships will allow us to predict the intensity of weed infestation and give us the opportunity to adapt control measures.

A field experiment was performed at the Mendel University agricultural enterprise in Žabčice, Czech Republic. Žabčice is located in the corn production area with a flat terrain and a long-term average annual temperature of 9.3 °C. Annual cumulative precipitation is 483.3 mm. The field trial was established in 2003 and covered an area of 2.3 ha (100 m x 225 m). Crop rotation was as follows: gourd alfalfa (*Medicago sativa* L.), alfalfa, winter wheat (*Triticum aestivum* L.), forage maize (*Zea mays* L.), winter wheat, sugar beet (*Beta vulgaris* L.), spring barley (*Hordeum vulgare* L.). Applied tillage variants were ; conventional tillage (CT), minimum tillage (MT) and no tillage (NT). Weed infestation was evaluated by numerical methods, where the number of weeds weas counted in each tillage variant of monitored growths on 1m<sup>2</sup> with 24 repetitions. Evaluation was conducted in winter wheat and spring barley, which are major field crops in the Czech Republic. Observations were carried out in the spring of 2004 and 2013, before herbicide application. Daily precipitation was recorded. Monthly rainfall totals were calculated from measured values for selected months (October to April). A multivariate analysis of ecological data, Canonical Correspondence Analysis (CCA), was used to determine the effect of precipitation on the weed species in winter wheat and spring barley.

Precipitation in the selected months significantly influenced the incidence of weed species in cereals. However, the response to rain and tillage differed between weed species. Higher amounts of precipitation in December favoured the occurrence of *Chenopodium album* L., *C. hybridum* L. and *Persicaria lapathifolia* L. Precipitation in December probably reduced seed dormancy of these species, resulting in more germination in the spring. Lower rainfall in February and March favoured weed infestation by *Medicago sativa* L. and *Galium aparine* L. Lower rainfall may limit the competitiveness of wheat and allow higher weed infestation of these species. Rainfall in November and January influenced the occurrence of *Veronica polita* Fries in spring barley, in all three variants of tillage. Rainfall in March encouraged *Cirsium arvense* (L.) Scop. in CT. Average or lower rainfall in February and March favoured its occurrence in MT. Higher amounts of precipitation in October favoured the incidence of *Fallopia convolvulus* (L.) Á. Löve in CT and NT. Lower rainfall in November and January supported this species in MT.

Studying the relationship between precipitation and the level of weed infestation requires longterm monitoring, which is time- and labour-demanding. Nevertheless, it may enable further development of weed infestation prediction.

#### SPATIO-TEMPORAL SHIFTS IN WEED PATCHINESS IN BARLEY

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Barley is one of the major crop species worldwide. It is used for stock feed, human food, malting, brewing and distilling. Although barley has high allelopathic potential, weeds can in some cases cause strong competition, which may lead to reduced yield or reduced malting quality. In order to investigate the weeds' continuous appearance or patchiness in relation to the allelopathic crop, a field site (1000 m<sup>2</sup>) with barley, free of herbicide applications, has been selected.

The experiment was carried out on AUA's experimental station (Spata), for three consecutive years (2012-2014). Intensive surveys of weeds were conducted over the course of 15 days during each growing period. In order to assess growth and spatial variability, the density and frequency of the weeds were recorded within 48 sampling units of 1m<sup>2</sup> separated by 3m following a regular grid sampling of 12 rows and 4 columns. We also recorded meteorological data, such as total precipitation and mean temperature. Spatial interpolation methods were applied and weed density maps for each sampling period for the three years were realized.

Weed density varied strongly between the three subsequent years, although the spatial weed appearance revealed the same pattern. In the first growing season, we recorded the following weeds: *Malva sylvestris* L., *Chamomilla recutita* L., *Sinapis arvensis* L., *Chrysanthmum coronarim* L., *Sillybum marianum* L., *Cardaria draba* L. and *Papaver rhoeas* L., in densities of 2.8, 1.8, 1.3, 0.5, 0.4, 0.4 and 0.2 weeds m<sup>-2</sup>, respectively. In the second growing period the densities of *C. recutita, Avena sterilis* L., *P. rhoeas, C. draba*, *M. sylvestris*, *C. coronarium and Convolvulus arvensis* L. were 3.3, 1.3, 0.9, 0.9, 0.8, 0.6, and 0.6 weeds m<sup>-2</sup>, respectively. Finally, in the third growing period we noticed the appearance of a new weed, *Lolium rigidum* L., occurring in high densities of 2.7 weeds m<sup>-2</sup>, while the densities of *A. sterilis, C. recutita, C. coronarium, P. rhoeas, C. arvensis* and *M. sylvestris* were 1.5, 1.4, 1.3, 0.7, 0.6 and 0.5 weeds m<sup>-2</sup>, respectively. Weed interpolation maps revealed that two of the most important species, *C. coronarium* and *M. sylvestris* developed different patchiness. Specifically, *C. coronarium* appeared in the same part of the field, whereas *M. sylvestris* was equally distributed. Ranking of species occurrence and density differentiated between the three years due to the dissimilar climatic condition of each year. In 2013, the combination of higher soil moisture with higher temperatures favored the emergence of *A. sterilis*, which changed the population dynamic of the other weeds.

Weed population have a varied patchy distribution. The temporal weed patchiness highlighted by interpolated maps indicates that weed appearance could have been affected by the seed bank. Weed maps may provide the necessary data for site-specific weed management by monitoring weed population dynamics.

#### CAN THE EFFICACY OF BROOMRAPE CONTROL METHODS BE HAMPERED BY INTER-AND INTRASPECIFIC VARIABILITY AS OBSERVED IN GERMINATION STUDIES FOR THREE BROOMRAPE SPECIES?

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Broomrapes are root holoparasites that infest a considerable number of crops causing significant yield losses worldwide. Parasitism constitutes a phenomenon that takes place underground and the host-crop is damaged prior to the emergence of the parasite. Because of this, not a single control strategy has been proven quite effective, economical and applicable and the results were sometimes inconsistent due to environmental variability. In addition, seeds produced by broomrape plants are characterized by long term persistence, which helps them to remain viable in the soil for decades. This provides the parasite with high genetic adaptability to environmental changes, which is expressed in physiological functions, such as germination. In most cases, control measures are applied against one or just a few populations of a broomrape species without considering intrapopulation variability. As a result, these control measures seem to be effective against one or a limited number of broomrape species, for specific crops under certain environmental conditions.

The study focused on elucidating the inter- and intrapopulation variability through the efficacy of Algit Super<sup>®</sup>, an aqueous solution of the algae *Ascophyllum nodosum* (L.) Le Jol., which induces seeds of three broomrape species to germinate. Petri-dish trials were conducted on 44 populations, namely *Phelipanche ramosa* (L.) Pomel. (18), *Phelipanche aegyptiaca* (Pers.) Pomel. (16) and *Orobanche crenata* Forsk. (10) at three different temperature (18, 20 and 23 °C). Three replicates were used per treatment. The populations were collected from naturally infested fields from eleven areas in Greece. *Phelipanche aegyptiaca* populations were sampled from tobacco crops, *P. ramosa* from tobacco and tomato fields, and *O. crenata* from three regions where host-crops (faba bean, peas and carrot) were present and parasitized. GR<sub>24</sub> was used as the stimulant control.

In most cases, broomrape seeds germinated to a greater extent after GR<sub>24</sub> compared to Algit Super<sup>®</sup> treatment. *Phelipanche aegyptiaca* and *P. ramosa* seeds responded much better to Algit Super<sup>®</sup> compared to *O. crenata*, which had a very low germination rate. In contrast, the radicle of all broomrape species was longer after Algit Super<sup>®</sup> application compared to GR<sub>24</sub>. High variability was observed between and within populations of all broomrape species for both germination and radicle length values. Germination ranged from 40-100%, 30-100% and 20-90% for seeds treated with Algit Super<sup>®</sup> at 20°C and from 50-100%, 40-100% and 70-100% after GR<sub>24</sub> treatment at the same temperature for *P. aegyptiaca*, *P. ramosa and O. crenata* seeds, respectively.

The study indicated that there was a high variability at the inter- and intraspecific level for all three broomrape species, a fact that constitutes a hindrance when considering only one strategy for broomrape control. In order to manage holoparasitic weeds more effectively and decrease the impact of population variability, more integrated approaches should be combined in a concerted manner.

#### ESTIMATION OF THE DEGREE OF SEED LIMITATION IN A WEED POPULATION IN MAIZE AND THE IMPORTANCE OF WEED SEED PREDATION

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Weed populations in arable fields are assumed to be mainly seed limited, *i.e.* dependent on the availability of seeds. This means that the addition of seeds leads to an increase in size of the weed population. In contrast, in populations that are microsite limited, population growth is determined by the availability of suitable microsites for seed germination and growth. The addition of seeds will not lead to an increase in population size.

This study investigates the extent of seed limitation of an annual arable weed and uses this information to determine the importance of seed predation for weed population growth. In each of three fields, 72 plots of 1.5 by 1.5 m were installed, half of them enclosed by 60 cm high plastic frames to prevent entrance of seed predators. Four different densities of *Echinochloa crus-galli* (L.) P.B. seeds (namely 300, 600, 1200 or 2400 seeds m<sup>-2</sup>) were sown into the plots, resulting in 12 plots per density and field, 6 with and 6 without a plastic frame. Beginning in spring 2015, the number of seedlings germinating from these seeds, and the surviving adult plants will be counted and accumulated over the next two years. These will be used as a measure of seed limitation. The difference in the degree of seed predation. We present and discuss the first results.

The funding of the project "Testing for the weed control potential of seed predators in agroecosystems" (WE 5040/2-1) is provided by the DFG.

## TROPHIC RELATIONSHIPS BETWEEN HOST AND PARASITIC PLANTS: A CASE STUDY WITH THE PARASITIC PLANT SPECIES *PHELIPANCHE RAMOSA*

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*Phelipanche ramosa* (L.) Pomel is the most frequent and harmful parasitic plant species in France. It reproduces not only on crop species but also on many families of weeds. Thus, even in the absence of a host crop, weeds can allow the increase of the *P. ramosa* soil seed bank, which may then infest a subsequent crop. The holoparasitic plant *P. ramosa* attaches to the roots of its host and extracts all its nutrients from the host. Consequently, host/parasite trophic relationships are crucial in determining the growth and seed production of the two plants interacting. The objective of the present study was to quantify the intensity with which *P. ramosa* draws carbon assimilates from its host and to analyze whether this intensity varies with (1) host species, (2) the phenological stage of the host plant, and (3) plant growth conditions.

A greenhouse experiment was conducted using three host species: oilseed rape (*Brassica napus* L.), the preferred *P. ramosa* host crop species and two host weed species, namely *Capsella bursa-pastoris* (L.) Medik. and *Geranium dissectum* L.. Plants of the three species were grown with or without *P. ramosa* seeds. They were grown under two contrasted light levels (100% and approximately 30% daylight) in order to evaluate the robustness of our results under contrasting growth conditions. Plant biomass was considered as a relevant trait to analyze carbon fluxes between the two plants interacting as *P. ramosa* extracts all its nutrients from its host. So, host and parasite biomasses were measured separately at four phenological stages of the host species, from rosette to fructification stage. Host biomass was analyzed in the presence and absence of *P. ramosa*. The biomass of *P. ramosa* was analyzed as a function of the biomass of its host.

Whatever the host species and the light level, the presence of *P. ramosa* significantly reduced the biomass of its host, with the total host+parasite biomass at a given date being lower than the biomass of the parasite-free host. The percentage of host biomass reduction due to the parasite showed that the negative impact of *P. ramosa* on host biomass increased during host growth and that the three host species differed in sensitivity to *P. ramosa*. Whatever the light level, *C. bursa-pastoris* and oilseed rape were, respectively, the least and the most sensitive host species. Whatever the phenological stage and the light level, the biomass of *P. ramosa* was the highest on oilseed rape, and this both in absolute value and relatively to host biomass. From our results, equations were proposed to predict both parasite biomass and host biomass reduction due to parasite as a function of host species identity, phenological stage and light level.

In the near future, results will be synthesized into a mechanistic model of the effect of cropping systems on pest dynamics. This model will help to design new strategies for the management of *P. ramosa* by identifying which techniques must be chosen and combined to optimize the control of both the parasite and susceptible weeds.

## CAN THE ATLANTIC SHRUBLAND COMPROMISE THE INVASIVE SUCCESS OF EUCALYPTUS GLOBULUS LABILL?

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On a global scale, invasive species are a huge environmental and socioeconomic problem. One example is eucalyptus (*Eucalyptus globulus* Labill). Scientific literature indicated that the atlantic shrubland is a dominant and supercompetitive community across the NW Iberian Peninsula. Preliminary field observations suggested that this type of shrubland is able to stop the spread of invasive tree species, including eucalyptus. Therefore, we studied the competitive interactions between two native species from the North Iberian Atlantic shrubland, namely *Ulex europaeus* L. (gorse) and *Cytisus scoparius* Link (broom), and eucalyptus.

We developed *in vitro* bioassays to test the effects of aqueous extracts and volatile compounds from fresh plant material of gorse and broom on the germination and seedling growth of eucalyptus. A greenhouse pot bioassay was conducted to assess early competitive interactions during seedlings establishment of shrubs and eucalyptus, using a replacement series.

Both aqueous extracts and volatiles from gorse and broom produced strong phytotoxic effects, as evidenced by reduced germination and early growth of eucalyptus seedlings. Compared to monocultures, the relative yields of eucalyptus seedlings decreased when grown together with shrub seedlings whereas shrubs seedlings are not affected by eucalyptus competition. This indicates the ability of the shrub species to exert competitive stress on eucalyptus.

Our motivation is try to find biocontrol methods for control or stop eucalyptus invasion.

Keywords: Invasive species, phytotoxicity, volatile organic compounds, competition.

#### FIELD STUDIES OF WEED POPULATIONS INFLUENCED BY GLYPHOSATE PRODUCTS

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Glyphosate products play a leading role worldwide in non-selective perennial and annual weed control in agriculture, but a growing threat of herbicide resistance calls for a better understanding of the effects of glyphosate products on weeds. Our hypothesis states that glyphosate products play a role in steering the composition of weed communities and the perpetuation of individual weed species. The current research employs three approaches to investigate the influence of glyphosate products on weeds.

The first approach focuses on monitoring weeds in practical agricultural fields to assess the response of weed communities to the repeated use of glyphosate products. Monitoring is restricted to winter wheat fields in the region surrounding Rostock in northeastern Germany in order to control for the influences of crop and location on the weed communities. A field monitoring consists of estimating weed species densities according to classes in three 2-by-50 meter randomly placed plots across a field. With the assistance of a hand-held GPS unit, the same plots in 51 fields were monitored in both the pre-harvest time period as well as in the stubble during the 2014 season. A maximum of five fields per farm are monitored in order to minimize the influence of farm management on the results. Farm records of fields allow for putting fields into glyphosate product use intensity groups: no herbicides, no glyphosate, low, medium, and high glyphosate.

The second approach is a field trial managing a naturally occurring weed community to assess the response of weed communities to the repeated use of glyphosate products in an experimental environment while comparing this chemical approach to the effects of mechanical weed management. The experiment is set up in a Latin square design (plots 5-by-6 m). Two of the four treatments include managing weeds chemically with an application of the recommended amount of glyphosate product (2.4 L/ha of 450 g/L a.i. glyphosate) and half of the recommended amount, while the two other treatments are the mechanical management methods of mouldboard ploughing and chisel ploughing. With the research goal of observing the effects of management over multiple generations, weed surveys followed by management with approximately 9-week re-growth intervals were carried out three times throughout the first research season and will continue in the 2015 and 2016 seasons.

The third approach is a field trial set up in randomized block design to assess the response of four selected weed species populations over multiple generations to glyphosate product treatments at reduced amounts from the recommended application. The selected species include *Arabidopsis thaliana* (L.) Heynh., *Lolium multiflorum westerwoldicum* Lam., *Papaver rhoeas* L., and *Senecio vulgaris* L. Dose response tests conducted in a greenhouse indicated which reduced amounts of glyphosate product would harm but not destroy each particular species. To account for possible fluctuations in response between greenhouse and field conditions, each species is subjected to two different reduced doses in the field trial. The assessment of population response to herbicide application is based primarily on the quantity and germination capacity of the seeds produced by each generation.

## ABOUT THE RISK THAT SEEDS OF WILD PLANTS USED FOR BIOGAS PRODUCTION ENTER THE BIOGAS-CHAIN

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In recent years, biomass production for generating biogas has strongly increased in Germany. For this purpose, most often maize or forage rye is ensiled and subsequently digested in biogas plants. Seeds of wild flower mixtures can provide an alternative substrate for anaerobic digestion. Depending on the species in the seed mixture, positive side-effects are expected, such as flowers for pollen- and nectar-feeding insects, shelter for wild animals, soil conservation, and aesthetic aspects for human beings. However, a risk is given that seeds of sown wild plants or unsown weeds will enter the biogas-chain, survive ensiling and fermentation, and will be spread over fields with the fermentation residues. Spreading seeds with the residues is undesirable because wild plants currently absent from the region until now may establish and become invasive. A further risk is the input of seeds of weed species into fields that have been free of this species so far.

The purpose of this study is to estimate the probability that seeds of a wild plant mixture are harvested together with the crop biomass and enter the biogas-chain.

In spring 2014 field trials were established at two locations in Northeastern Germany with different soil quality. The commercial wild plant seed mixture Biogas 1/BG 7 (Saaten Zeller), developed for biogas production, was sown. Biogas 1/BG 7 consists of 24 species. The wildflower mixture is expected to remain in the field for several years, with most biomass coming from annual plants in the first year, from biennial plants in the second year, and from perennial plants in the following years. One treatment used the recommended sowing density, and one only 2/3 of the recommended density to simulate poor establishment. Field trials were laid out as a block design with four replicates. At the time of harvest on 18th September 2014, 1 m<sup>2</sup> of each plot was sampled. Seeds of the sown and weed species that had not shed at harvest were counted. Viability of the seeds was tested in sub-samples.

Data of the first year of field trials with mainly annual species will be presented. Species with high input of viable seeds will be identified. The results will allow to quantify the risk that seeds of sown species and of the accompanying weeds will enter the biogas-chain.
#### CAN SOWING SELECTED FLOWERING PLANTS IN THE FIELD MARGINS SUPPORT WATERMELON POLLINATION?

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Watermelon has unisexual flowers and relies solely on insect pollination. The reduction of indigenous flowering plants, offering food and shelter to pollinating insects in intensively cultivated areas, is expected to have a negative effect on the production of insect pollinated crops. This study assessed the capacity of selected flowering plant species established next to a watermelon crop to attract pollinating insects that could visit and pollinate the crop.

The experiment was established in Chania, Crete. Two mixtures of selected plant species were sown on 29 November 2013: *Borago officinalis* L., *Coriandrum sativum* L., *Chrysanthemum coronarium* L., *Scabiosa atropurpurea* L., *Crepis vesicaria* L. (mixture A) and *Vicia sativa* L., *Sinapis alba* L., *Phacelia tanacetifolia* Benth., *Fagopyrum esculentum* Moench, *Matricaria chamomilla* L. (mixture B). Each mixture was sown in four plots and covered an area of 48 m<sup>2</sup>. Another plot of equal size containing natural vegetation without soil disturbance was allocated as the control. Watermelon plants were transplanted on 17 April 2014 in approx. 200m<sup>2</sup>. Natural vegetation between crop rows (70m<sup>2</sup>) served as second control (vegetation after soil disturbance). Crop plants were drip-irrigated and received fertilizer according to their needs. Flowering per species (%) and numbers of attracted Hymenoptera pollinators based on counts of insect-visits per flower, were measured biweekly in the sown plants, natural vegetation (control plot and inter-row space) and crop flowers.

Sown species that established successfully and reached flowering were *C. coronarium* > *B. officinalis* > *C. sativum* (mixture A) and *V. sativa* > *S. alba* > *P. tanacetifolia* (mixture B), listed in order of flowering. The main flowering species of the natural vegetation was *Eruca sativa* Mill, emerging in high density between crop rows. It also emerged and flowered among the sown plants. Flowering of sown plants lasted from early April to end of May, while flowering of watermelon plants started mid-May. Mixture B attracted the highest number of *Apis mellifera* L. (honeybee) during flowering of *P. tanacetifolia* and *S. alba*, while mixture A attracted on average the highest number of mining bees during flowering of *C. coronarium*. Other pollinating species included *Eucera* sp. On *V. sativa* and *Anthophora* sp. on *E. sativa*. The control plot had low flower cover and the numbers of honeybees and mining bees during synchronous flowering of the crop, sown field margins and natural vegetation (mid-May) while number of honeybees was smaller. *Eruca sativa* flowers attracted high numbers of honeybees at the end of May. Subsequently, *E. sativa* plants were removed and a temporary increase of honeybees on the crop flowers was recorded.

The results of this study suggest that the presence of selected plant species near insect pollinated crops, such as watermelon, can attract increased numbers of pollinating insects and thus could enhance the pollination of the crop. However, synchronous flowering of other species with a watermelon crop may have a distraction effect for certain pollinators.

This work was funded under the biodiversity project Operation Pollinator of Syngenta.

# EXPLORING THE LIFE-HISTORY STRATEGY OF THE FACULTATIVE ROOT PARASITIC WEED RHAMPHICARPA FISTULOSA

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Rhamphicarpa fistulosa (Hochst.) Benth is a parasitic weed species in large areas of rainfed, low land rice fields of sub-Saharan Africa (SSA). The species has been a minor weed until recently, when rice cultivation expanded into marginal wetlands, the natural habitat of the weed. In contrast to other parasitic weeds of rice, such as *Striga asiatica* (L.) Kuntze and *S. hermonthica* (Del.) Benth, it is facultative, meaning that it can complete its life cycle in the absence of a host. For obligate parasitic weeds, like *Striga* spp., stimulation from root exudates is a prerequisite for germination while seeds of *R. fistulosa* do not need the presence of a host to germinate. However, the ability to germinate in the absence of a host does not exclude an enhanced germination when a host is available. Particularly, since association with a host represents a major fitness advantage for parasitic plants. It is feasible that a germination stimulatory mechanism can be selected for through evolution.

Greenhouse and laboratory experiments were conducted to establish whether root exudates of the rice host plant stimulated seeds of *R. fistulosa* to germinate. In a randomized complete block design (60 pots; 5 replicates) with rice cultivars IAC 165, IR64 and Shiokari, known to be high, medium and low strigolactone producers, emergence of *R. fistulosa* did not differ from that in control pots without rice. Root exudates collected from the same cultivars and a solution of artificial germination stimulant GR24, were added in three concentrations to Petri-dishes containing seeds of either *R. fistulosa* or *S. hermonthica*. Germination of *S. hermonthica* correlated strongly with strigolactone concentration but did not germinate in water (control). *Rhamphicarpa fistulosa* showed no relation with strigolactone concentration in that germination in all solutions was similar to that in the control (water).

Two weed population models were constructed to investigate whether *R. fistulosa's* nonresponsiveness to the presence of host root exudates is a superior strategy. In the 'responsive' model host presence was set as a condition for germination, whereas in the 'non-responsive' model germination was made independent of host presence. In both models, a set of life cycle characteristics and environmental variables were systematically varied. The 'non-responsive' model generated higher population equilibria than the 'responsive model under high seed mortality, low seed germination rate, low ability to attach to a host, small fecundity advantage of being connected to a host, a small host range and few host plants present. The ecological implication is that, for facultative parasitic plants, absence of a mechanism to synchronize germination with the life cycle of a host is not necessarily a disadvantage. This opportunistic life-history strategy of facultative parasitic plants has also implications for weed control. Opposite to what is commonly found for obligate parasitic weeds, strategies based on trap crops will not be effective, whereas stale seedbeds suddenly bare promise.

## HOW ARE RARE ARABLE WEEDS DESCRIPTORS OF LOW FARMING INTENSIFICATION?

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Recent emphasis has been placed on the rapid destruction of the planet's biodiversity in all habitats. Rare arable weeds, also known as segetal plants, are threatened species, mostly in decline due to intensive farming systems. However, the presence of these species is intrinsically linked to agroecosystems. Their survival is apparently related to low-input agronomic practices. Could these weeds therefore be good descriptors of low agricultural intensification and be good indicators of high environmental field value? We propose to examine the relationships between the farming systems and weed flora sampling in the same fields.

Several fields with contrasted levels of intensification (organic, integrated and conventional) in different regions of France were sampled in 2013 and 2014. In each field, weed flora at three contrasted field positions (boundary, edge and center of the field) and agronomic practices (intensity of chemical control, nitrogen supply, farm type, etc.) were recorded. Weed communities were described by species richness and frequency of occurrence for common weed species, as well as for subgroups that are recognized for their conservation value. Subgroups of weed species were based on the Red List, either regionally, nationally, or as 'messicoles' (i.e. segetal species).

At the farm level, the number of species present in the various subgroups are highly positively correlated. But, they are not correlated with total species richness. For all the subgroups, the species richness are negatively correlated with agronomic practices mainly the intensity of chemical control. These subgroups thus appear as good indicators of low farming intensification.

#### **BIODIVERSITY-BASED ECOSYSTEM SERVICES IN VINEYARDS**

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Vineyards may establish and maintain a high degree of biodiversity and include rather complex ecosystems, especially if they are situated on steep slopes with small-scale terraces. Within this agroecosystem, the land-sharing vs. land-sparing debate is in principle less of an issue, as areas between vine rows could host a diverse flora of local species or cover crops. Traditionally, landscape elements such as solitary trees, hedges and stone walls are also important elements of diverse temperate viticultural agroecosystems. These elements also provide important ecosystem services including erosion mitigation, habitat and food sources for natural enemies and also fruits. In addition, biologically and structurally diverse viticultural landscapes provide a high aesthetic value for recreation and tourism. However, these multifunctional viticulture agroecosystems are declining, due to more intensified and increasingly mechanized management. Current agrienvironmental regulations may additionally promote the eradication of structures that favor biodiversity, such as trees or stone walls, within vineyards since these areas reduce the area eligible forsubsidies.

Traditional vineyard management was characterized by frequent tilling in order to eradicate all spontaneous plants ('weeds') and to reduce water and nutrient competition between vines and such weeds. Later, herbicides were frequently used for removing these weeds. These management measures are nowadays associated with ecosystem disservices such as very high rates of erosion, degradation of soil structure and fertility, contamination of groundwater and high levels of agricultural inputs such as pesticides. These and other management measures (*e.g.* application of copper fungicides) have also been shown to negatively affect earthworms, important ecosystem engineers and the largest component of the soil faunal biomass.

Detailed studies are currently still lacking on the effects of species-rich inter-row vegetation on a variety of ecosystem services in different viticultural landscapes. Even if cover crops are increasingly becoming the state of the art in vineyard management in areas with summer rain or irrigation, common seed mixtures of legumes/grasses might suppress the former typical vineyard bulb geophytes and therophytic species. Plant biodiversity therefore not only provides essential supporting and regulatory ecosystem services such as the conservation of soil structure and fertility, nutrient cycling and clean water provision, but also food source and habitat for beneficial arthropods and pollinators. Our present knowledge on the interactions between the different trophic levels and associated ecosystem services is still very poor and therefore it is impossible to predict how these interlinkages will be altered by changed land use and climate change. In the BiodivERsA project VineDivers, we will analyze the implications of different management regimes in vineyards (high intensity tilling regimes vs. 'green' vineyards with cover crops or wild plants) on above- and below-ground biodiversity and the associated ecosystem services. Within each region two different vineyard management regimes will be applied in low and high diversity landscapes. To be able to test at which scale these interactions work, data will be collected at the plot, vineyard and landscape scale in each region across Europe (Spain, France, Austria and Romania).

# EFFECT OF TILLAGE ON THE VERTICAL DISTRIBUTION AND DIVERSITY OF WEED SEEDS IN THE SOIL

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The goal of weed control is not only to prevent yield loss, but also to minimize weed seed reserves in the soil, because the soil seed bank is the primary source of new infestations of annual weeds. It was established that shallow ploughing and rototilling resulted in an increase in weed incidence. The aim of present study was to evaluate the influence of different soil tillage intensities on diversity and vertical distribution of seeds in the soil seed bank.

Experiments were conducted in 2003 on a cultivated field, with sandy loam soil, at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry,  $(55^{0}2350)$  N and  $23^{0}5140$  E). Soil tillage treatments were: CT, conventional tillage; RT1, reduced tillage 10-12 cm deep and sowing without predriling soil cultivation RT2, reduced tillage 10-12 cm deep and sowing with prie-drilling soil cultivation NT1, no tillage and sowing of the aggregate without predrilling cultivation; NT2, no tillage and sowing the aggregate with a rotary cultivator. The design was a complete randomized block in four replicates. Soil samples to determine the soil seed bank were taken in 2011 at the beginning of the growing season. Two soil cores of 20 cm depth (0-5, 5-10, 10-15, 15 - 20 cm) were randomly taken from each plot, using a 5cm diameter steel probe. Consequently, a total 480 soil samples were collected for weed seed bank analysis.

A total of 17 weed species were found in the seed bank; 98 % were annual dicotyledons. *Chenopodium album* L., *Lamium purpureum* L., *Tripleurospermum perforatum* (L.) <u>Sch.Bip.</u> and *Stellaria media* (L.) Vill. composed more than 80 %of the total amount of weed seeds. The high densities of these weeds in the seed bank indicate possible severe infestations if future weed management is inadequate. Other weed species of importance in the seed bank were *Veronica hederifolia* L., *Viola arvensis* Murray and *Galium aparine* L.

The amount of seeds in the soil was significantly higher in less disturbed systems, particularly in the no-tillage system. No significant differences in the size of weed seed bank were obtained between reduced and no-tillage systems. The distribution of weed seed over different soil layers was more even in the conventional tillage system. Compare to 0-5 cm, significantly lower amounts of weed seed were found in soil layers 10–15 and 15–20cm in the reduced and no tillage systems.

Understanding the impact of management practices on the vertical distribution of seeds is important as it can help us predict weed emergence patterns. The higher amount of seeds in the upper soil layer indicate that weed numbers in future crops may increase and reducing weed seed return in systems with less disturbed soil is important, in order to reduce the need for weed control in the future.

#### INFLUENCE OF P OR N FERTILIZATION ON WEED SEED GERMINATION

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Predictive emergence models have been developed for certain weed species, such as *Chenopodium album* L. and *Conyza bonariensis* (L.) Cronquist. None of these models consider the possible effect of the level of fertilization. The goal of this study was to investigate the effect of different phosphorus or nitrogen rates on germination of a collection of Mediterranean weed species.

Two experiments were conducted under controlled conditions with a completely randomized design. In the first experiment, 42 species were included, with 10 treatments differing in  $KH_2PO_4$  or  $KNO_3$  rates. Based on this experiment, 26 species were selected and the number of treatments was extended to 16. In all cases, the experimental unit was a 5.5 mm diameter Petri dish filled with 8 ml of water-agar solution to which the appropriate dose of  $KH_2PO_4$ , or  $KNO_3$ , was added, plus 20 weed seeds. Within each experiment, 2 petri dishes were included for each species and treatment. The first experiment was repeated twice ( $42 \times 10 \times 2 \times 2 = 1680$  plates) and the second was repeated 3 times ( $26 \times 16 \times 2 \times 3 = 2496$  plates). Germination was rated every 24 hours for 11 days. As both P and N nutrients were added in formulations with potassium, the K concentration was equilibrated by adding KCI. With this equilibration, all P treatments had the same K concentration (including the control), and the same applies to N treatments. In the second experiment, an additional control was included without KCI, to test the possible effect of KCI on the germination process.

Comparisons between controls showed that KCI did not influence the germination. Also, added P or N did not affect germination in most species. But in some cases, interesting results were found. Added P increased the germination rate in Diplotaxis virgata (Cav.) DC. and decreased it in Chrysanthemum coronarium (L.) Cass. ex Spach. In the case of C. bonariensis, glyphosate resistant (GR) biotypes had lower germination rates than susceptible ones (GS), at all P rates. Added P increased the germination rate in the GS biotype, whereas it decreased in the GR biotype. GR and GS biotypes were also included for C. canadensis (L.) Cronquist but yielded opposite results compared to those obtained for C. bonariensis. For C. album, a Spanish population and a Danish population that had been included in a collaborative study (EWRS Germination and Early Growth WG) were included. As in the collaborative study, the Danish population had a higher germination rate than the local population. Added P increased the final germination rate in both C. album populations. Added N increased the germination rate in the 3 Amaranthus species studied (Amaranthus albus L., A. blitoides S. Watson and A. retroflexus L.) and in Rumex crispus L.. On the contrary, added N reduced the germination rate of Plantago coronopus L., Abutilon theophrasti Medik. and Digitaria sanguinalis (L.) Scop. In the case of Centaurea diluta Aiton, added N did not affect the final emergence rate, but caused a significant delay in germination.

# A FUNCTIONAL APPROACH TO TEST THE RESPONSE OF WEED COMMUNITIES TO AGRICULTURE INTENSIFICATION ACROSS TWO COUNTRIES

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Agricultural intensification at local and landscape levels has been the main cause of weed diversity decline and changes in weed community composition in agro-ecosystems. There is evidence that this has occurred through a filtering effect on the weed flora, with intensification selecting against species with certain attributes. Because of decreased inputs of fertiliser and herbicide at field edges, they have been recognised as important refugia for weeds from these selection pressures. Edge communities could, therefore, be functionally discrete, being more similar to weed floras pre-intensification when compared to the centre of fields.

To test the hypothesis that the gradient of management intensity, at the within and between field scales, is reflected in a shift in the functional composition of weed floras, a trait-based approach was used to compare weed communities in 29 conventionally managed dryland cereal fields located in Spain (established vegetation and seed bank) and 95 in the UK (established vegetation). The seed bank was assessed in 23 out of the 29 sampled fields in Spain. Functional traits were life form, growth form, plant height, SLA, LDMC, Seed dispersal type, seed mass, flowering onset and flowering duration. The variables reflecting intensification at the within field scale were crop cover, margin width, margin management and distance from the margins and at landscape level were field size and arable land cover. To test the relationship between functional traits and intensification variables, RLQ analysis and fourth-corner analysis were employed separately on each dataset.

No statistically significant relationships between the distribution of specific species traits and intensification variables were detected in any of the three datasets, except proportions of eudicotyledons which were lower in large fields in the Spanish established vegetation. This was despite the fact that species richness and functional diversity were higher at the edge of fields in both datasets. We suggest that the conventional management system that has been carried out for several decades in both areas has been the main environmental filter modulating community assembly in the two datasets and any remaining gradient of intensification is not sufficient to drive a functional shift in weed communities at the within field scale. The mean and range of trait values are quite similar in the datasets, suggesting a convergence in the trait values of the weed communities in response to intensification. The similarity in trait values between established vegetation from its seed bank.

#### HIGH IMPORTANCE OF INTRASPECIFIC TRAIT VARIABILITY IN WEEDS IN RESPONSE TO CLIMATE AND MANAGEMENT CHANGES

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In weed ecology, the usefulness of trait-based approach has been emphasized since two decades and increasingly applied to investigate the processes that govern weed assembly. The rapid expansion of large trait databases has facilitated the application of this approach. However, the use of values from databases is not always the most relevant choice, especially because it implies averaging values over multiple populations and habitats. For studying within-community processes, *in situ* measurements appear necessary to capture the intraspecific variability observed in many weed species.

In a field experiment located in north-eastern France, we investigated weed functional diversity in arable fields characterized by different two-year crop sequences, i.e. analysing both the influence of the crop type – winter wheat, winter oilseed rape and spring pea – and its preceding crop type on the functional structure of weed communities. First, we focused on the relative contribution of intra- and interspecific variability of four key functional traits involved in response to management practices and resource acquisition processes: specific leaf area (SLA), leaf dry matter content (LDMC), plant lateral spread (PLS) and canopy height (CH). In addition, considering that weed communities are composed of a few abundant and numerous subordinate species, we focused on the three most abundant species to investigate intraspecific trait variability in response to two-year crop sequence types, crop-weed competition and growing season conditions during their life cycle.

The relative contribution of intra- and interspecific variability among the two-year crop sequence types was highlighted using an approach based on diversity partitioning of the Rao guadratic entropy metric. The analysis provides evidence for substantial amount of intraspecific variability in weed community for all traits, and especially for SLA, accounting for more than 75 % of intraspecific variability in the four two-year crop sequence types. This result questions a key tenet of community ecology assuming that trait values vary more between than within species and stresses that the intraspecific variability is important when studying changes in weed community in arable fields. At the community-scale, we also highlighted the role of the current and the preceding crop type on weed trait distribution, the latter appearing mainly explained by the different proportions of autumn-germinating individuals, besides related to the sowing date of the crop. The results of linear models suggest a concomitant influence of the crop sequence types, crop-weed competition and growing season conditions on weed trait distributions. However, crop-weed competition – here estimated using median crop CH, a reliable proxy to assess competitive ability for light - more frequently explained trait variability across 'species × traits' combinations. Interestingly, crop CH highly explains LDMC values for the three most abundant species, suggesting for a niche differentiation mechanism driven by resource partitioning among weed shaded plants. Trait-based approach using in situ measurements constitute a relevant tool for understanding weed assembly both by taking into account multiple influential factors characterizing the agroecosystem and by incorporating intraspecific trait variability.

# THE ADAPTATION OF CORNFLOWER IN DIFFERENT COMPARTMENTS OF THE AGRICULTURAL LANDSCAPE

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Agrosystems are among habitats most affected by the decline of biodiversity. This decline is related to the intensification of agricultural practices carried out since the 50s (simplified rotations, fertilizer and pesticide use; ...). The study of the weed flora shows still less developed plant communities. Currently, there is evidence of the regression of the weed flora and especially species such as cornflower (*Cyanus segetum*. L). In western Europe, cornflower seems to be present in the field only. In our investigation, we tried to determine the factors that influence the presence of cornflower in the different compartments of a field and its border.

Cornflower seeds were sown in four field compartments: boundary (B), crop edge (CE), field margin (FM) and field core (FC)) of two winter crops (wheat and mustard) in the fall and spring. Morphological and phenological measurements were done. No weeding was made during the experiment.

The establishment rate of cornflower is higher for autumn than spring cohorts. The plants from spring cohorts have implemented fewer leaves than those from autumn cohorts a harvest time. The cornflower has a higher emerged rate in crop edge for mustard and in field margin for wheat. Emerged cornflowers in crop edge of fall mustard and in field margin of wheat have the highest biomass/height ratio. Plants establishing at the spring must complete their cycle so faster, it would explain at least partially their lower biomass. Cornflower in field margin and field core of wheat and in crop edge of mustard are the most developed plants. The plants present in crop edge are more branched than those in the others compartments. Generally, plants in the boundaries are less developed than those of the other compartments.

Therefore, in general, the success of cornflower in an agricultural landscape is partly related to the interface area that could be considered a safe haven for cornflower with low competition with other plants and with less efficacy of herbicides. Parameters of cornflower concurrence against the crop are currently under study.

# **SESSION IV**

# **INTEGRATED WEED MANAGEMENT**

### TOWARDS A CONTROL STRATEGY AGAINST CYPERUS ESCULENTUS L. IN SWITZERLAND

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*Cyperus esculentus* L. (yellow nutsedge) is one of the most problematic weeds in many crop production systems in subtropical and tempered zones around the world. It is often mentioned as an invasive species. The species was found in Switzerland 1989 as troublesome weed in the Ticino region, belonging geographically to the North Italian range. Its presence in the Swiss Mittelland region north of the Alps was reported for the first time 1992. Recently "Infoflora.ch" listed yellow nutsedge on the so called 'black list' as a plant species which must be controlled because it has the potential to harm. In pots the tuber production rate/year was 1:747; germination rate in the greenhouse was >95%.

Two identical field trials were carried out on farm in two different sites – mineral soil and organic soil – in two subsequent years. No crop was grown at any time; soil tillage in spring replaced seed. Weeds were controlled by the test compounds and application methods. The field experiments were set up as a two factor randomized split-plot design with herbicide application method as the whole-plot factor: pre-plant incorporation = PPI (tillage immediately after herbicide application) and herbicide surface application =SUR and 11 herbicide compounds (+ untreated) as the sub-plot factor and four replications per treatment. Plots untreated with herbicides served as control.

Soil samples were taken at both trial sites in March 2012 for initial tuber infestation levels. This sampling was repeated in autumn 2012/13 and in spring 2013/14 to evaluate efficiency of applied herbicides (PPI and SUR). Herbicide PPI application leads to an overall decrease in yellow nutsedge tuber formation in both soil types studied. SUR application of herbicides strongly reduced their efficacy and lead to an overall increase in tuber formation in both soil types. Statistical analyses were performed with R 3.0.1 using RStudio to evaluate differences between the whole plot factors of the two soil types (MS and OS) and the two treatments (PPI and SUR) as well as differences between initial tuber numbers and the following counts.

Shoot number was determined in the 2-6 leaf stage in spring 2013 and 2014. The linear correlation between the number of shoots/0.25m<sup>2</sup> and the number of tubers/L soil in the different trial locations was pooled over the two years. We could observe that the number of tubers present in the soil and the number of sprouts growing from the tubers are weakly to moderately correlated.

When rhizomes were destroyed mechanically by repeated tillage, tuber numbers decreased strongly. However, tuber numbers were even lower after PPI of the following herbicides: flazasulfuron, halosulfuron, sulfosulfuron and s-metolachlor. Weed competition was estimated visually once in both years and found to have an impact in certain conditions.

From the experience of these field trials we conclude that best yellow nutsedge control is achieved by combining herbicidal activity, soil tillage and crop/weed competition.

#### SENSITIVITY OF ECHINOCHLOA CRUS-GALLI POPULATIONS TO MAIZE HERBICIDES: A COMPARISON BETWEEN CROPPING SYSTEMS

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*Echinochloa crus-galli* (L.) P. Beauv. is an important maize weed with huge variation in herbicide sensitivity. This differential response may reflect differences in selection pressure caused by years of cropping system related herbicide usage. The herbicide sensitivity of *E. crus-galli* populations from three divergent cropping systems was evaluated in dose-response pot experiments. Populations were collected on sandy fields with (1) a long-term organic cropping system, (2) a conventional cropping system with maize in crop rotation or (3) a conventional cropping system with long-term monocropping of maize. Each cropping system was represented by six *E. crus-galli* populations. The effectiveness of three foliar-applied maize herbicides (S-metolachlor and dimethenamid-P) was tested at three doses. Foliar-applied herbicides were applied at the three true leaves stage. Soil-applied herbicides were applied immediately after sowing. The foliage dry weight per pot was determined four weeks after treatment. Plant responses were expressed as biomass reduction.

Herbicide sensitivity was consistently lowest for populations from maize monocropping systems. Compared to populations from organic cropping systems, populations from monocropping systems were 5.3, 5.7 and 12% less sensitive to cycloxydim, topramezone and nicosulfuron respectively. Populations from the conventional crop rotation system showed intermediate sensitivity levels. Sensitivity to dimethenamid-P and S-metolachlor was not affected by cropping system. Environmental conditions influenced herbicidal response except for nicosulfuron. Integrated weed management may be necessary to preserve herbicide efficacy on the long term.

#### ON-FARM EVALUATION OF INTEGRATED WEED MANAGEMENT TOOLS IN MAIZE PRODUCTION: AGRONOMIC EFFICACY, HERBICIDE USE REDUCTION AND ECONOMIC SUSTAINABILITY

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The development and implementation of integrated weed management (IWM) strategies that provide good weed control while reducing dependence on herbicides, and preferably without having side effects on the overall system economic performance, is still a challenge that has to be met. To achieve this and promote IWM implementation, robust evidence on the sustainability of such strategies is needed to motivate their adoption by stakeholders. This can only be done through assessing and validating them at real farm scale and using existing farm equipment, under diverse climatic and soil conditions that represent the reality of European agriculture.

In 2011 and 2012, within the European Project PURE (Pesticide Use-and-Risk reduction in European farming systems with Integrated Pest Management, http://www.pure-ipm.eu), nine on-farm experiments (i.e. real field conditions on commercial farms, with natural weed flora) were conducted in three important European maize producing regions-countries, which represent the range of climatic and edaphic conditions in Europe, to evaluate the efficacy of different locally selected IWM tools for direct weed control in maize vs. the conventional approach (CON) followed by the farms. The IWM tools tested were: 1) early post-emergence herbicide band application combined with hoeing followed by a second hoeing in Southern Germany, 2) early post-emergence and scouting in the field, followed by hoeing in Northern Italy and 3) tine harrowing at 2-3<sup>rd</sup> leaf stage of maize and low dose of post-emergence herbicide in Slovenia. A further comparative assessment was performed to identify herbicide use reduction and economic sustainability of the IWM tools tested against CON management.

Results showed that the IWM tools tested in the different countries: (1) provided sufficient weed control without any significant differences in yields, (2) greatly reduced maize reliance on herbicides (i.e. Treatment frequency index reduced as an average across countries from 1.9 in CON to 0.8 in IWM), (3) costs of IWM ranged from -82 (in Italy) to +32 (in Germany)  $\in$  ha<sup>-1</sup>, however (4) IWM implementation was economically sustainable as no significant differences in gross margin were observed in any country compared to CON. It was highlighted that IWM in Europe, and IPM as a conceptual framework, is not about a unique weed control strategy but is based on general principles that must be adapted to address specific local agro-environmental and social conditions.

#### WHAT ARE THE *REAL* EFFECTS OF HERBICIDE USE ON WEED CONTROL AND CROP YIELD IN FARMERS' ARABLE CROPS?

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Until recently, weed control has mainly relied on herbicide use. However, herbicide use has eventually raised much societal concern with regard to its environmental consequences, since it generates strong negative impacts on ground and surface water. It has also a strong impact on biodiversity leading to the decline of many taxa. Finally, the spread of herbicide resistance in many weed species makes these species more difficult and expensive to control. Consequently there has been recently a general call for reducing the use of herbicides. However, herbicides use reduction raised a major question: since herbicides are implicitly supposed to improve crop yields, will herbicide use reduction result in a decrease of crop yield through the indirect effect of weed abundance increase? Answering this question requires knowledge on the relationship between herbicide use and crop yield. However, and paradoxically, even if the potential loss of crop yield due to weeds has been estimated at 34%, quantitative data of weed effects on crop production are limited. Weed damage thresholds are known, but these approaches considered only individual weed species, and have not included the compounding of effects at the community level. Moreover they did not integrate the influence of agricultural practices and environmental variables on the weed-crop relationship. These limitations weaken the prediction of potential crop yield losses due to weeds in cropping systems with low herbicide input.

Here we aim to quantify the relationship between crop yield, herbicide use intensity and weeds. Hidden Markov models with a latent variable were used to explain and quantify crop yield loss due to weeds over a wide range of agronomic situations, by analyzing weed samples, crop yield and farming practices in 150 arable fields in the LTER "Zone Atelier Plaine & Val de Sèvre" collected in 2010. Crop yield and weed richness (abundance) are variable, and this variation can only be partly explained by agricultural techniques. Indeed, this variation also comes from the intrinsic variability of weed-crop competition output and from the efficiency of weed control practices. The link between weed richness and crop yield was performed through hidden Markov model to account for the variations in efficiency of these agricultural practices.

No significant effect of herbicide use was observed on crop yield nor on weed richness or abundance. The efficiency of herbicide use was shown to highly vary between farmers but also between farmers' fields. Overall, our results showed that contrary to what could be expected, herbicides were more efficient to control rare weed species than abundant species or those which are usually perceived as pernicious. In conclusion, to the question "will herbicide use reduction result in a decrease of crop yield through the indirect effect of weed abundance increase?", our empirical study seems to answer that weed richness could increase through the presence of rare and non abundant species, and that crop yield may not be significantly affected.

#### EFFECT OF HERBICIDE DOSE, ROTATION AND CROP COMPETITION ON MANAGEMENT OF CROSS-RESISTANCE TO PRE-EMERGENCE HERBICIDES

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In the Australian wheatbelt herbicide-resistant *Lolium rigidum* (Gaud.) (ryegrass) is a major constraint to grain yield and quality. At present pre-emergence herbicides represent the benchmark to control ryegrass plants resistant to selective post-emergence herbicides (i.e. group A and B herbicides) as well as the rapidly escalating problem of glyphosate resistance. Currently, Australian grain growers highly rely on pre-emergence herbicides such as trifluralin and propyzamide (Group K1), prosulfocarb (Group N) and pyroxasulfone (Group K3) to control multiple-resistant ryegrass in small grain cereals crops. For prosulfocarb and pyroxasulfone no resistance has been reported from the field and the herbicide propyzamide (group K1) has been recently registered for use in canola.

Since the original study by Neve and Powles (2005) subsequent studies have shown the danger of cutting herbicide rates. For resistance-prone weed species like ryegrass, low rates of herbicide (i.e. cut below the recommended label) enables the genetic diversity in ryegrass to become evident and survivors of the cut rate of herbicide can rapidly evolve resistance. This has been shown in several AHRI studies with pot cultured plants as well as in field conditions (Busi *et al.*, 2014; Busi *et al.*, 2012; Busi and Powles, 2013a; Manalil *et al.*, 2011; Neve and Powles, 2005).

We will present our results starting from a study conducted before the commercialization of a new herbicide (pyroxasulfone) in which we observed rapid pyroxasulfone resistance evolution in a ryegrass population by low-dose selection over three generations (Busi et al., 2012). We demonstrated that pyroxasulfone low dose selection resulted in cross-resistance to prosulfocarb and triallate (Busi and Powles, 2013b) and this ryegrass population is also resistant to trifluralin (Busi et al., 2014). We must emphasize that no ryegrass populations have thus far been reported to be resistant to pyroxasulfone or prosulfocarb. In a subsequent Mendelian inheritance study we showed that resistance to different herbicides segregates at single loci (Busi et al., 2014). Recent studies show that continuous selection with pyroxasulfone followed by prosulfocarb may lead to genetic drift in small ryegrass populations (Busi and Powles, unpublished). We will present the results of long term modeling simulations with the polygenic platform PERTH (Polygenic Evolution of Resistance To Herbicides) and discuss the effect of herbicide dose, combinations of herbicide options (rotation) and competition from a crop to formulate scenarios for optimal control of ryegrass and maximal delay of cross-resistance to important pre-emergence herbicides such as trifluralin, prosulfocarb, propyzamide and pyroxasulfone. Herbicide diversity within a set of integrated management tactics can be an important component to reduce the herbicide selection intensity.

#### THE EFFECT OF SEED RATE AND HERBICIDE PROGRAMME ON ALOPECURUS MYOSUROIDES IN HYBRID BARLEY AND WHEAT

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Alopecurus myosuroides (Black-grass) is the major weed problem in the UK and populations with resistance to a wide range of herbicides are ubiquitous. The arable industry is starting to realise that control of this pernicious weed by herbicides alone is unsustainable and that a reversion to integrated non-chemical control is unavoidable. Changing the crop grown can be an effective method for controlling *A. myosuroides*, hybrid winter barley is known to be more competitive against weeds than winter wheat.

Hybrid winter barley cv Volume and winter wheat cv JB Diego was sown at two seed rates on 23 Sept 2013 at ADAS Boxworth, Cambridgeshire, UK on a clay soil of the Hanslope series. The hybrid winter barley was sown at 200 and 250 seeds/m<sup>2</sup> and winter wheat at 300 and 375 seeds/m<sup>2</sup>. There were three herbicide treatments; no herbicide pre-emergence herbicide only or a pre and post emergence herbicide programme. The trial was a fully randomised factorial design (Species x seed rate x herbicide programme) with three replicates.

*A. myosuroides*, barley and wheat plants were counted in November and number of heads in June. In July, ten *A. myosuroides* plants were sampled from each plot. Each plant was separated into viable and non-viable tillers. Seed was removed from each head, dried and weighed. Seed number per plant and seed number per tiller were calculated using a thousand seed weight of 2.5 g. All data was subjected to ANOVA. The trial established well and *A. myosuroides* was present at populations up to 217 plants/m<sup>2</sup> in the autumn with up to 439 heads/m<sup>2</sup> in June.

The results showed that there were no differences in *A. myosuroides* populations in the autumn between the hybrid barley and the wheat, but the *A. myosuroides* plants in the hybrid barley produced 61% fewer heads in June. Individual *A. myosuroides* plants in the hybrid barley produced 43% fewer tillers and 65% less seed per plant than those in winter wheat. There was 91% less *A. myosuroides* seed return between hybrid winter barley and winter wheat sown at a high seed rate and receiving a full herbicide programme. The trial is being repeated this year at multiple sites in the UK.

### AN ANALYSIS OF THE EFFECTS OF COVER CROPS' COMPETITION STRATEGIES ON WEED GROWTH

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Cover crops (CC) can contribute to the biological control of weeds via competition for resources. CC integration in cropping systems could be an alternative to the exclusive use of herbicides but the issue is to identify CC species or combinations of CC species adapted to local requirements. We previously developed a plant trait-based approach, originated in functional ecology, which allowed us to distinguish three competition strategies based on architectural and physiological plant abilities for seventeen potential CC species for banana cropping systems. The objective of the present study was i) to assess the efficiency of ten CC species with different competition strategies for the control of a weed species, *Bidens pilosa* (*BP*)- and ii) to evaluate if this efficiency differ between an early and a late control (timing thereafter).

A field experiment was conducted with BP and ten CC species. We tested three competition strategies (shading, obstruction or smothering) \* two levels of control efficiency (+ or -, based on aboveground biomass) \* two timings of control (early or late). Species with "shading" strategy only have the ability to shade weeds. Species with "obstruction" strategy have the ability to shade and they have adventitious roots on twigs which can obstruct the growing space of weeds. Species with "smothering" strategy have the ability to shade and they have tendrils enabling them to climb and smother weeds. A field experiment was conducted with 10 independent plots of monospecific cultures of BP as control treatments and 55 independent plots of weed-CC mixtures. We chose an additive design to evaluate the interspecific interactions. The CC density was determined for early (resp. late) control considering that CC species cover the ground twice quicker (resp. slower) than weed. The effects of the different CC species on BP were evaluated by their impact on BP growth traits. For light competition, we measured aboveground dry biomass, height, span, Specific Leaf Area (SLA), Leaf Dry Matter Content, Leaf Area Ratio, Leaf Mass Fraction, Leaf Soil Area ratio and chlorophyll leaf indicator. For soil-resources competition we measured root biomass, Specific Root Length and Root Dry Matter Contents from root samples at three depths. The experiment duration was 50 or 110 days according to the timing of control.

A preliminary analysis was based on Kruskal-Wallis tests to compare means of plant trait values between monospecific cultures of weed and weed-CC mixtures on the early control modality. Results suggest that the traits of *BP* which most respond to CC are height, SLA and chlorophyll measure and that the + level of each strategy has a stronger effect on *BP* than the - level. Among the three CC strategies, "smothering" and "obstruction" caused the most of changes on the weed traits.

A deepest analysis will allow us to make assumptions on the links between the traits defining strategies and the effects observed on a target weed in order to improve biological weed control by CC.

#### LONG TERM EFFECTS OF COVER CROPS ON WEEDS IN MEDITERRANEAN LOW INPUT ARABLE MANAGEMENT SYSTEMS

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The introduction of cover crops (CC) in crop rotations is a key tool to control weed and ameliorate soil conditions in low input arable systems. In 1992 a long term experiment (LTE) was set up at *Centre for Agri-environmental Research "E. Avanzi* (CIRAA), University of Pisa to determine the combined effect of tillage intensity, nitrogen fertilization levels and CC types on soil quality, crop yield and weed communities.

The LTE is based on 4-year crop rotation (maize, durum wheat, sunflower, durum wheat) with cover crops grown twice, in between wheat and summer crops. Two tillage systems were compared: i) a conventional system (CS), with annual ploughing (30 cm depth); ii) a low input system (LIS), with chiseling (30 cm depth) for summer crops and no till for wheat. Four Nitrogen levels (from 0 to a maximum rate, varying across crop type) were applied. Cover types were: control; *Brassica juncea* L.;*Trifolium squarrosum* L.; and *Vicia villosa* Roth.). The experiment was replicated in 4 blocks for a total of 128 plots (21 x 11 m). The experiment is arranged as split-split plot design with tillage system in main plots, nitrogen dose in sub-plots and cover crop type in sub-sub-plots. Weed and CC above-ground biomass were assessed at CC termination, while weed species cover and total biomass were assessed at harvest. Data collected in CC (2011, 2014), sunflower (2012), durum wheat (2012) were used to calculate species richness, weed community diversity indices (Shannon's H' and inverse Simpson index) and Pielou equitability. Weed biomass and cover were analyzed by split-split plot ANOVA. Weed community composition was analyzed by a permutational multivariate analysis of variance based on Bray Curtis dissimilarity and Nonmetric Multidimensional Scaling.

During both CC cycles, CC type significantly affected weed composition, biomass and species richness. Compared to the control, cover crops significantly reduced weed biomass with different effectiveness depending on CC type.

In sunflower, weed species richness at an early stage was higher after *T. squarrosum* whereas no difference due to CC effect were recorded at harvest. Tillage system and nitrogen dose significantly affected weed community composition but not diversity. In durum wheat, weed community composition at harvest was significantly affected by tillage system and nitrogen dose but not by cover crop type, unlike weed biomass and cover.

CC type strongly influenced weed community composition during CC cycle but this effect was barely detectable in summer and winter cash crops. LIS favored mainly the presence of perennial weeds, increasing also weed total biomass compared to CS. This suggests that some adjustments in CC management under LIS may be needed to prevent potentially troublesome weed shifts which might offset the benefits attained by reduced tillage systems on other production-related agroecosystem services.

### COVER CROPS IN POTATOES AS AN ALTERNATIVE ENVIRONMENTALLY-FRIENDLY MANAGEMENT PRACTICE

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The use of cover crops (CCs) as a soil conservation management practice is attractive because of CC benefits in multiple agronomic aspects. Most CCs belong to three botanical families: grasses, legumes and crucifers which suppress weeds through competition on light, water and minerals and by exuding allelopathic toxins. In addition CCs reduce soil erosion and water runoff, especially in highly inclined sandy soils, by shielding and adhering the soil during heavy rains. Furthermore, CCs reduce water evaporation from soil and decrease extreme temperatures during potato cultivation.

The aims of this study were: i) To examine the most efficient management of common oat (*Avena sativa*) as a winter cover crop before planting of winter potatoes, and ii) To evaluate several termination practices and their effect on weed suppression, soil erosion, soil temperatures and potato yield and quality.

A field study was conducted in 2013-2014 on a sandy soil commercial plot in Moshav Mishmeret in the central Mediterranean coastal plain of Israel. Common oat as a CC was seeded at the end of October 2013. Each treatment consisted of three 300 m long adjacent beds. The experiment design was randomized blocks with four replications. CC termination treatments were: 1) Mowing. 2) Roller crimper 3) No mechanical treatment. 4) No CC on commercial ridges. All CCs were killed with glyphosate + carfentrazone ethyl three weeks before potato (*var.* Sifra) seeding. Periodic weed counts and identification, soil moisture content, soil temperature and light intensity were recorded, and finally potato yield and quality of the different treatments were estimated.

All CC treatments reduced weed infestation with no need for mechanical tilling compared to the no CC control, which required rototilling before potato planting. CC prevented heavy soil erosion compared to the no CC control and reduced extreme soil temperatures at 5 cm depth so that in the winter max temperature was 3°C higher and 6°C lower in the summer.

Soil water content in the no CC control plots at potato emergence was highest, and lowest at the end of the crop. There was no reduction in yields or yield quality in the CC treatments compared to the commercial no CC on ridges control. CC management was found to be an environmentally-friendly tool to manage multiple agronomic aspects of potato production.

Studies are in progress to test the possibility of further reducing herbicide application and reducing irrigation in an oat CC regime compared to a no CC commercial regime.

# EFFECT OF CONSERVATION AGRICULTURE ON WEED INFESTATION IN RAINFED RICE

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It has been argued that the practice of Conservation Agriculture (CA) provides an agro-ecological approach to reduce weed pressure on crops. However, very few data are available on the effective impact of CA on weed infestation, especially in tropical countries. In this study we quantified the effect of CA principles (no-tillage, mulching and crop rotations) on weed infestation in rainfed rice in Madagascar.

Emergence and biomass of weeds and grain yield of rice were determined during the 2013-2014 growing season in two experiments that were carried out in Ambatondrazaka, Madagascar. In experiment A, two types of mulch were applied in a rice crop under no-tillage: *Stylosanthes guianensis* (S) and maize + *Dolichos lablab* (MD), using different amounts of crop residues (0, 0.9, 3.2, 12, 18.3 and 0, 1.4, 4.8, 18.4, and 27.5 t ha<sup>-1</sup> for S and MD mulch, respectively). In experiment B, two types of crop rotation were studied both under conservation (no tillage and residue retention) and conventional (tillage and no residue retention) agriculture: a two-year rotation of MD and rice, and a three-year rotation of maize + *S. guianensis*, *S. guianensis* and rice. For both experiments weed emergence was measured every week from December 2013 to March 2014, and weed biomass was determined before weeding (four and three times on experiment A and B respectively).

In experiment A, weed emergence decreased with increasing mulch quantity following a decaying exponential function. Weed biomass in treatments with respectively 0.9 and1.4 t ha<sup>-1</sup> of S and MD mulch did not differ significantly from that in treatments without mulch. Plots with respectively 3.2 and 4.8 t ha<sup>-1</sup> of S and MD mulch showed half the weed biomass of that of plots without mulch. Weed biomass was close to zero for treatments with amounts of mulch higher than 12 t ha<sup>-1</sup>. Rice grain yields increased with the amount of mulch in the case of S, whereas there was no difference in rice yields with different amounts of MD mulch. In experiment B, weed emergence and biomass were lower with three-year rotation than two-year rotation both under conventional and conservation agriculture. For a given rotation, weed emergence and biomass were lower under conventional agriculture.

Results showed that conservation agriculture was effective to reduce weed infestation, especially at the beginning of rice cycle when soil was not totally covered by the rice vegetation, with no or positive effect on rice yield according the type of mulch. However, a high amount of mulch is required to control weed emergence and growth. To confirm these results, additional experiments are ongoing.

#### WEED EMERGENCE AND POPULATION DYNAMICS IN ALTERNATIVE IRRIGATION SYSTEMS IN CALIFORNIA RICE

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Rice in California is largely continuously flooded, primarily for control of certain weed species. Recently, due to unprecedented drought, concerns about water usage have increased and alternative irrigation methods have been proposed. Little is known about implications of such methods on weed emergence and population dynamics. The primary objectives of this research were to quantify: 1) differences in field emergence patterns of *Cyperus difformis* L. (smallflower umbrella sedge) and *Echinochloa phyllopogon* (Stapf.) Koss (synonym *E. oryzicola* Vasinger; late watergrass) under dry and flooded conditions; and 2) species-specific relationships for major weed species between percentage cover at canopy closure, biomass at harvest, and yield loss under alternative irrigation systems.

All studies were conducted at the Rice Experiment Station, in Biggs, CA, in 2013. Emergence was counted in two fields with populations of *C. difformis* and *E. phyllopogon*. The experiment was a Randomized Complete Block Design (RCBD) with three replications per irrigation treatment: 1) Drill-seeded alternate wet and dry (DS-AWD): drill-seeded, then flush-irrigated each time volumetric water content (VWC) of the soil reached 35% (approximately –2.0 MPa); and 2) Water-seeded conventional (WS-Control): flood of 10-15 cm, drained one month prior to harvest. Daily counts of emerged seedlings were conducted in three 25 cm<sup>2</sup> quadrats per replication. Plants were removed at each count until no more plants emerged (canopy closure of the rice).

To assess population-level dynamics, three irrigation systems were evaluated in an RCBD with three replications per irrigation treatment: 1) DS-AWD; 2) Water-seeded alternate wet and dry (WS-AWD): flooded for initial seeding through canopy closure of the rice, allowed to drain, then flushed each time VWC reached 35%; and 3) WS-Control. At canopy closure, visual assessments of relative cover of major weed species (*Echinochloa* spp., *C. difformis, Leptochloa fusca* spp., *Schoenoplectus mucronatus* (L.) Palla, *and Ammannia* spp.) were taken from an herbicide-free section of each treatment. At harvest, measurements of fresh and dry biomass per-species were taken from the same untreated areas. Rice was harvested from two 18.5-m<sup>2</sup> areas in the weedy and weed-free sections of each treatment.

The total number of *C. difformis* seedlings emerged from DS-AWD was less than emerged from WS-Control (ANOVA, p < 0.005). Similar numbers of *E. phyllopogon* seedlings emerged from DS-AWD and from WS-Control (ANOVA, p > 0.05). *E. phyllopogon* emerged under both aerobic and anaerobic conditions, making it competitive regardless of irrigation system. *C. difformis* emerged at high numbers under flooded conditions, but was not competitive against other species after emergence. Low *C. difformis* numbers in DS-AWD suggest temporary moisture stress as a potential cultural control method. Yields were not different across treatments when weeds were fully controlled with herbicide, but without weed control, yields were significantly less in the DS-AWD (Tukey's HSD,  $\alpha = 0.05$ ). Grasses (*Echinochloa* spp. and *L. fusca* spp.) were the drivers of yield loss across all irrigation systems. Grasses out-competed rice, sedges and broadleaves. In the presence of grasses, sedges and broadleaves did not contribute significantly to yield loss.

#### ASSESSMENT OF TEMPORARY GRASSLAND IMPACT ON WEED FLORA IN FOLLOWING CROPS

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The insertion of temporary grasslands can be an effective tool to control weed flora in annual crop rotations. The aim of the present work is to evaluate the effect of temporary grasslands in weed abundance and diversity in the following crops. To do this, we observed weed flora dynamics in the long term Observatory and Experimental System for Environmental Research - Agroecosystems, Biogeochemical Cycles, and Biodiversity (SOERE-ACBB) at the INRA research centre of Lusignan (Poitou-Charentes, France). We compared weed flora in five treatments differing in temporary grassland duration (0, 3 or 6 years) and N fertilization (260 kg N.ha<sup>-1</sup> vs. 30 kg N.ha<sup>-1</sup>). Seed bank was measured every three years.

In previous studies we showed that weed abundance decreased in temporary grasslands according to their duration and N fertilization. Weed diversity was also changed resulting in a more balanced weed flora composition with less harmful species. We observed changes in seed bank density and composition throughout the six-year rotations but less important than in weed flora.

Our hypothesis is these seed bank modifications will influence weed flora in the following crops. We will compare weed flora dynamics in maize/wheat/barley rotations differing in preceding conditions (different grassland duration and N fertilization). We observed no differences in crop yields for maize and wheat whereas barley yields were significantly increased following rotations with six-year old grasslands. Furthermore, we quantified a maximum of 36% reduction of herbicides use due to the insertion of temporary grasslands in the crop rotations. This study will give a quantitative assessment of temporary grassland impact on the change in abundance and composition of weed communities in the following annual crops. We also will be able to evaluate the relationship between weed flora observed in crops and the seed bank composition.

### MULTIPLE BENEFITS OF DELAYED DRILLING FOR ALOPECURUS MYOSUROIDES (BLACK-GRASS) CONTROL IN WINTER WHEAT

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In many parts of North Western Europe, rotations of predominately winter sown crops are under threat from herbicide resistant black-grass. Due to increasing resistance to post-emergence herbicides (ALS and ACCase inhibitors), growers are placing greater reliance on pre-emergence residual chemistry and, to a lesser extent, implementing cultural control options. The aim of this project was to measure the impact that drilling date has on emerging *A. myosuroides* plants, their fecundity and the efficacy of residual herbicides.

In 2010, 2011 and 2012, five field trials were set up which included 3 target drilling dates (mid-September, early October and late October), and +/- a non ALS herbicide programme in a split plot design in randomized blocks. The mid-September drilling dates ranged from 16/9 to 21/9, 3/10 to 11/10 for the early October target and 21/10 to 5/11 for the late October date. The pre-emergence herbicide programme (flufenacet+diflufenican (240+60 g a.i. ha<sup>-1</sup> + prosulfocarb (1600 g a.i. ha<sup>-1</sup>) was applied 7-14 days after drilling. A mixture of clodinafop (30 g a.i. ha<sup>-1</sup>) and prosulfocarb (2400 g a.i. ha<sup>-1</sup>) was applied early post-emergence. Surviving black-grass plant numbers were assessed and inflorescence counts carried out at the end of May every year, with heads collected to calculate fecundity.

There was considerably variation in the population sizes between years and sites on all trials. Delaying drilling without a herbicide programme significantly reduced black-grass plant and inflorescence numbers in 4 out of the 5 trials. Where the herbicide programme was included, there was a significant reduction in black-grass plant and inflorescence numbers where drilling was delayed on all 5 trials. Control from the pre-emergence programme increased with delayed drilling, averaging 47% (1 to 74%), 73% (45 to 97%) and 70% (51 to 88%) control from the successive drilling dates. Pre-emergence herbicide efficacy was consistently better with delayed drilling in all trials. The number of black-grass inflorescences per plant decreased with delayed drilling. A black-grass plant in the mid-September drilled plots produced on average 15.4 inflorescences per plant, compared with 7.9 and 5.5 on the October drilled plots.

These trials demonstrate three distinct benefits from delaying drilling, by 3 weeks; 1) there were fewer black-grass plants emerging in the plots drilled in October, compared to mid-September. This is due to the greater time for destroying plants pre-drilling. 2) The efficacy of residual herbicides not only increased with delayed drilling, but the level of control was more consistent. This was probably a consequence of greater soil moisture resulting in better control from the residual herbicides. 3) The black-grass plants that emerge in later drilled crops are less competitive and therefore produce fewer inflorescences per plant than those plants that come up in a mid-September sown crop. The outcomes remained consistent, regardless of climatic conditions. Producing data sets like this, with clear concise outcomes that show the benefits of cultural control is all well and good, but the difficulty is trying to get the land users to change their habits. The challenge is to change the mind set of farmers, because carrying on relying on herbicides for black-grass control is not sustainable long term with resistance increasing. An integrated approach, as demonstrated here, is vital.

#### HOW TO CONTROL WEEDS IN ARABLE FARMING? ANALYSIS OF TECHNICAL DRIVERS IN A NATIONAL DEMONSTRATION FARM NETWORK FERMECOPHYTO

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Weed control remains a critical aspect of crop production in agriculture. Here we propose to assess the potential benefits of prophylactic and curative agronomic measures in weed management. Our analysis is based on the technical data that have been collected from more than 200 different cropping systems implemented between 2011 and 2013 in commercial farms as part of FERMEcophyto - Réseau des Coopératives (cooperatives network). These farms are representative of the diverse French production situations.

The aim of this study was to discuss the effect of weed management strategy on cropping system performances. Various weed management practices were studied such as ploughing, sowing date shifting, crop rotation diversification, mechanical weeding and false seed bed. We assessed the consistence of these practices by computing a range of performance indicators, and above all productivity, herbicide use, profitability, workload, and weed resistance risk. Those indicators were analyzed at the annual and field scale as well as at the cropping system scale.

Among other results, this study pointed out that crop rotation had a major effect on weed management and pesticide use. For instance, wheat preceded by spring crop results in a higher gross margin. Besides, cropping systems with both winter and spring crops are less reliant on herbicide and they also display a higher gross margin than winter crops based cropping systems. Concerning ploughing, it seemed that no-till farming systems use more herbicides than farms with ploughing annually. However, there was no herbicide use variation depending on ploughing frequency. The sowing date study highlighted that winter wheat sown lately had a lower herbicide use compared with winter wheat sown early or at normal timings. But this went with lower yield and gross margin. Furthermore, for wheat, there was no effect of false seed bed on herbicide use and yield. This may be due to the fact that this practice has a long term effect rather than a short term one. The last studied practice was the introduction of mechanical weeding in a conventional herbicide program (mixed weeding) for maize. Fields where this practice has been implemented have a lower herbicide use and higher yield but also higher mechanical costs.

This study underlined that some agronomical practices that show good results under experimental conditions are also effective in commercial arable systems. On the contrary, some others are not so good considering farming system or annual field scales.

# THE COMPETITIVE ABILITY OF CHICKPEA (*CICER ARIETINUM*) AGAINST WILD MUSTARD (*SINAPIS ARVENSIS*) UNDER NON-IRRIGATED CONDITIONS

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Globally, chickpea (*Cicer arietinum* L.) is an important legume used for human feed, while it is often included in crop rotation schemes. Its poor competitive ability against the weeds due to its relatively small height and growth rate is among the main problems for its organic production. Wild mustard (*Sinapis arvensis*) is among the most noxious and troublesome weeds for chickpea and other winter legumes.

The main objectives of the present study were to evaluate the competitive ability of chickpea against *S. arvensis* and to determine the effects of timing of weed removal and duration of weed interference on growth, seed yield and biomass production of chickpea.

A field experiment was conducted in Domokos region in Central Greece in 2013. Chickpea cv. Amorgos was seeded according to the common practices on 25 February, 2013. The experimental area was naturally infested mainly with wild mustard and less with some *Poaceae* weeds. The experiment was arranged in a completely randomized design with four treatments (based on presence or absence of *S. arvensis*) and four replicates. Plot size was 2 x 4 m. Treatments were consisted of weed-free plots, weedy plots (with wild mustard being the main weed) and plots with wild mustard plants interfering with chickpea for the 3 first weeks after emergence (WAE) or from 3 to 6 WAE. Weed removal within and between crop rows was carried out by hand hoeing during the crop growth. Crop growth measurement were taken weekly throughout the experimental period, while at harvest several parameters such as plant height, root length, number of pods, seed yield and biomass production were also determined.

Our results revealed that the presence of wild mustard throughout the crop growth cycle resulted to growth reduction and up to 54 and 34 % reduction of seed yield and biomass production, compared with the weed-free plots, respectively. It also needs to be noted that this intense competition resulted in deficient establishment and lower crop density in the weedy plots. Regarding the two other treatments, our results showed significantly higher yields in the case of a weed-free crop for 3 WAE compared with the plots in which wild mustard plants were hand-removed after 3 WAE.

The length of weed-free period implies that under the non-irrigated conditions of Greece, chickpea is very susceptible to the competition imposed by wild mustard and if only one hoeing treatment can be done, this should be done very early in order to keep the field weed-free especially during the 3 first crucial weeks of its life.

# COMPETITIVENESS OF FOUR SUNFLOWER HYBRIDS AGAINST THE INVASIVE WEED SOLANUM ELEAGNIFOLIUM CAV.

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Sunflower (*Helianthus annuus* L.) is a widely grown oil crop, lately expanding in more semi-arid Mediterranean regions. Weeds, and especially the broadleaf species, can cause substantial yield losses to sunflower. Among them, the invasive species silverleaf nightshade (*Solanum elaeagnifolium* Cav.) turns to be one of the most noxious weeds for many crops, however its effects have not been systematically and widely quantified.

A field experiment was conducted in Agricultural University of Athens in order to evaluate growth and grain yield of four sunflower hybrids (Neoma, Cllever, PR64HE39, PR64LL62) under competition with silverleaf nightshade. The hybrids were seeded by hand in 75-cm rows on 2 May 2014 to achieve an approximate density of 70,000 plants ha<sup>-1</sup>. The experimental design was a split-plot in a randomized complete block with three blocks (replicates). Sunflower hybrid was the main plot factor and the weed presence (or total and partial absence) was the subplot factor. Main plot size was 6 by 4 m. Plant emergence began 6 days after sowing and was completed within 9 days after sowing. Weeds were controlled during the growing season by several cultivation and hand-hoeing treatments, while in the partial absence of *S. eleagnifolium*, this was permitted to grow only after two weeks after sowing. Irrigation and other common cultural practices were conducted as needed during the growing seasons.

Five measurements of plant height, leaf number, leaf area, plant biomass and inflorescence weight were taken during the growing period for sunflower, while grain yield, weight of 1000-grains and grain number were recorded at harvest on 26 August 2014 for all hybrids and treatments. All data were analyzed by ANOVA and means were separated by Fisher's Protected LSD test at a significance level of p = 0.05. Our results revealed that most growth characteristics were significantly affected by weed competition but this was depending on the hybrid. The presence of silverleaf nightshade seems to delay the flowering of sunflower, especially in hybrids Neoma and PR64LL62. Moreover, regarding grain yield and biomass the differences ranged from 3 to 18 % and from 11 to 34 % between the weedy and weed free plots for the several sunflower hybrids, respectively. PR64HE39 was the most productive hybrid, despite its low first growth rate. In our experiment, Neoma was the most competitive hybrid against *S. eleagnifolium*, since there were not any significant reduction in the grain yield of the weedy compared to the weed-free plots.

The results of the present study indicated that the growth and yield of sunflower hybrids might have a substantial range in terms of their weed competitive ability and should be certainly taken into account for the crop establishment in each region, especially when weeds such as silverleaf nightshade are present and herbicide applications are not possible.

# EFFECT OF PERIOD OF WEED INTERFERENCE ON GROWTH AND YIELD OF MAIZE (ZEA MAYS L.) IN THE SOUTHERN GUINEA SAVANNA OF NIGERIA

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A field experiment was conducted at the Teaching and Research Farm of the Kwara State University during the 2013 cropping season to determine the effect of critical period of weed interference on maize growth and yield. The trail consisted of 10 treatments. One set of treatments consisted of plots initially kept weed free for 3,6,9 and 12 weeks after sowing (WAS) and subsequently kept unweeded until harvest. The second set of treatments consisted of plots initially left weedy for 3, 6, 9 and 12 weeks after sowing and subsequently kept weed free till harvest. Also, there were two control plots, one left unweeded till harvest and the other kept weed free till harvest. The above treatments were laid out in randomized complete block design (RCBD) and replicated three times. Parameters measured were weed dry weight, maize plant height, leaf area, cob weight, 100-seed weight, and grain yield. Data collected were subjected to analysis of variance and where 'F' was significant, means were separated using Duncan's Multiple Range Test (DMRT).

Results showed that weed interference beyond 6 WAS significantly depressed all the crop growth parameters and maize grain yield compared with crop left weedy for only 3 WAS and the one kept weed free for 6 WAS and until harvest. In order to obtain maize yield comparable to that of weed free till harvest, it is required to keep the crop weed free between 3 and 6 WAS.

# OROBANCHE (OROBANCHE SPP.) AND PHELIPANCHE (PHELIPANCHE SPP.) IN LENTIL (LENS CULINARIS MEDIC.); IMPACTS ON YIELD, QUALITY AND MARKETING PRICES

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This study aimed to assess the impacts of severe parasitic plants (*O.crenata, P.ramosa, P. aegyptiaca*) on to grain yield, some quality characteristics and marketing price of red lentil in the South-east Anatolia. Farmer field trials were carried out in the Adıyaman and Viransehir locations in 2010-11 and 2011-12 crop growing seasons respectively.

Randomly distributed twin plots throughout the field relaying on required number of broomrape infestation in Adıyaman field trial in 2010-11 (1+ 1 m<sup>2</sup> each) as experimental units were nominated as follow;  $A_0B_0$ : control,  $A_1B_1$ : 30-39 broomrape flowering in twin plots;  $A_2B_2$ : 40-49 broomrape flowering twin plots;  $A_3B_3$ : 50-59 broomrape flowering plots and  $A_4B_4$ : over 60 broomrape flowering plots. (4 treatments) Twin plots (2 replications) in Viranşehir in 2011-12 (1+ 1 m<sup>2</sup> each) as experimental units were nominated as follow;  $A_0B_0$ : control,  $A_1B_1$ : 2-3 broomrape flowering in twin plots;  $A_2B_2$ : 4-5;  $A_3B_3$ : 6-7 and  $A_4B_4$ : 8-9 broomrape flowering plots. Grain yield, hectoliter weights and 1000 kernel weights were scored. All grain samples were presented to randomly chosen grain purchasers in local commodity market and marketing price offers were scored respectively.

Results revealed that broomrape infestation under both low and high levels of epidemic reduced the grain yield ranging from 51.5% to 97.7% significantly. Some visual purchasing criteria such as hectoliter and 1000 kernel weights were not affected seriously. Purchasers offered very similar marketing prices for pulse grains with all severity levels. Economic losses were huge varying from \$396.77 to 678.50 ha<sup>-1</sup> It was concluded that regression equations derived from grain yield vs. infestation densities were found to be reliable with high coefficients of determinations and can be perfectly used for yield estimates under various levels of broomrape infestations.

## TRAITS RELEVANT FOR CROP-WEED COMPETITION IN SINGLE STANDS AND VARIETAL MIXTURES OF COMMON WHEAT

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Developing crops more competitive against weeds is a major challenge for organic crop breeding as weed suppressive ability is a highly demanded agro-ecosystem service in organic and low input cereal production. Crop competitive ability is the result of a combination of phenotypic traits that, especially during the early stages of crop development, ensure crop advantage over weeds, e.g. early vigour, soil cover, and crop canopy height. It is known that variation in weed suppressive ability exists among wheat cultivars. However, a single variety cannot be expected to possess all the traits that can contribute to competitive ability against weeds. Therefore, the objective of our work is to test whether wheat competitive ability can be enhanced by the use of varietal mixtures.

We investigated the competitive ability of common wheat (*Triticum aestivum* L.) against the infestation of one dicot (*Sinapis alba* L.) and one monocot (*Lolium multiflorum* Lam.) species in an outdoor pot experiment. We used six wheat cultivars. Three were the modern varieties Altezza, Artico and Bramante, short, very productive, belonging to the biscuit making quality group. The other three varieties were Autonomia A, Gentil Rosso and Verna, tall, low productive old varieties that were cultivated in Central Italy before the development of modern cultivars. Every single variety, the mixture of the three modern varieties, the mixture of the three old varieties and the mixture of the six varieties altogether were grown with a mixed infestation of *S. alba* and *L. multiflorum* in a RBD design with four blocks. We used 30 X 30 cm wide pots filled with peat soil for a depth of 30 cm. Weed density was adjusted to 250 plants m<sup>-2</sup>, while wheat density was adjusted to 400 plants m<sup>-2</sup> both in infested and weed-free pots. The development of crop and weeds was monitored from emergence to beginning of wheat stem elongation phase (BBCH 30). At this stage, total crop and weed biomass were measured. Crop and weed data for phenology, chlorophyll content, N uptake and biomass accumulation were collected in 2013/14 and 2014/15. Data were analyzed by year with ANOVA and using Tukey HSD as post-hoc test.

Plants showed different growth patterns in the two growing seasons. In 2013/14 emergence and growth was slower compared to 2014/15 because of adverse weather conditions. In 2013/14 cv. Gentil Rosso and the three component mixture including it reduced biomass accumulation, N uptake and tillering in *L. multiflorum* as well as chlorophyll content and N uptake in *S. alba*. These results were not confirmed in 2014/15. Results are discussed focusing on understanding the relative importance of functional identity, i.e. traits possessed by the single varieties, and functional composition, i.e. combination of traits in the mixtures, in enhancing the weed control agroecosystem service.

# EFFECT OF CROP PLANTING PATTERN AND NITROGEN APPLICATION METHOD ON WEED CONTROL IN CHICKPEA

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Weeds are a major problem in chickpea because of its relatively low competitive ability. In addition, there are no efficient herbicides available to control weeds in chickpea. Therefore, searching for non-chemical options to suppress weeds is of important priority in chickpea production. Methods of nitrogen application and crop sowing arrangement can play an important role in suppressing weeds. Foliar application of nitrogen has been considered as an alternative method of fertilizer application to improve crop growth and reduce nitrogen input in crop production systems. Therefore, the aim of the present study was to investigate the effect of foliar application of liquid nitrogen and crop sowing arrangement on control of weeds in chickpea.

A field experiment was conducted to study the effect of sowing arrangement and method of nitrogen application on control of weeds in chickpea in Khoram Abad, Lurestan, Iran in 2012 -2013. The experiment was conducted in a randomized complete block design with split plot factorial arrangement and with three replicates. The crop sowing arrangement (including single, twin equidistance and twin triangular rows) were assigned to the main plots and nitrogen application methods (including control, broadcast, strip and foliar application) and weed control were assigned to subplots. The crop density was held equal (55 plant/m<sup>2</sup>) in all treatments. Chickpea yield, biomass, 100-seed weight, height, productivity index, seed per plant, double seeded pod, single seeded pot, aborted pod, pod per plant, nod per main stem, first pod height, numbers of primary and secondary branches, and weed density and biomass were measured.

Results showed that sowing arrangement significantly affected the yield, biomass, 100-seed weight, height, productivity index, nod per main stem, pod per plant, single seeded pod, and weed density and biomass. The present study showed that the highest crop yield was achieved in twin triangular, twin equidistance and single row sowing arrangement, respectively. Thus, twin triangular row was found best to increase chickpea yield (1858 kg/ha), among all crop sowing arrangements. Nitrogen application method significantly affected all measured crop variables except first pod height and number of primary branches. Foliar application of nitrogen significantly increased the crop yield and it also increased weed density and biomass, in comparison to other methods of nitrogen application. Weed density and biomass were lower in the twin triangular and highest in single row crop arrangement. Weed biomass and density were favored by foliar nitrogen application.

It is concluded that foliar application of nitrogen may favors weeds more than the crop in mixture. In this case nitrogen should be applied directly on crop rows. In addition, proper crop sowing arrangement seems to be a promising option to suppress weeds. Therefore, crop sowing arrangement can be considered as part of integrated weed management system to control of weed in chickpea.

#### WEED-CROP INTERACTION: INFLUENCE OF FERTILIZATION ON EARLY GROWTH

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Weed communities are supposed to adapt to predictable agricultural practices such as fertilization. Thus, fertilization should be taken into account in integrated weed management programs. Nevertheless few studies have explored this approach. The goal of this study was to determine the influence of phosphorus and nitrogen fertilizer on weed-crop interaction during the early stages of development.

An experiment was conducted in a growing chamber under controlled conditions. The experimental design was completely randomized, with 11 species (including two crops), 8 fertilizer treatments, and 3 replications. One weed plant was planted with one crop-plant in siliceous sand and irrigated with Hoagland type solution with variable nitrogen and phosphorus concentrations depending on the fertilizer treatment. Each weed-crop couple, seeds were germinated in petri dishes with filter paper and distiled water. Individual seedlings of each couple were transplanted to pots of 55 mm diameter and 73 mm deep and 200 g of pre-washed siliceous sand. Winter weed species were: *Anthemis mixta, Centaurea diluta, Chrysanthemum coronarium, Conyza bonariensis* and *Lolium rigidum*, and were grown with a durum wheat plant. Summer weed species were: *Abutilon theophrasti, Amaranthus retroflexus, Chenopodium murale* and *Echinochloa crus-galli* were grown with a sorghum plant. Fertilizer treatments involved 4 nitrogen rates (0, 50, 100 and 200 mg/L in the nutrient solution) and 4 of phosphorus rates (0, 0.5, 1 and 2 mg/L). The parameter studied was fresh and dry biomass at 28 days after seedling transplanting.

No influence of nitrogen fertilization on weed-crop interaction was observed; however, weeds showed different responses to phosphorus fertilization; in crops, responses to phosphorus supply depended on the companion weed in the pot.

For winter species, phosphorus fertilization increased the biomass in all Asteraceae weed species studied. Furthermore, the presence of *C. diluta* or *C. coronarium* increased the positive response of wheat to phosphorus fertilization. And similar, but smaller, effect was found in *A. mixta* and *C. bonariensis*. On the other hand, *Lolium rigidum* did not affect wheat development.

Overall, studied weed summer species did not influence crop (sorghum) response to phosphorus fertilizer. Only, *A. theophrasti* caused a slight positive response in the crop. *E. crus-galli* and *C. murale* showed a positive response to phosphorus fertilization. Surprisingly no response was detected to phosphorus fertilizer in species adapted to high input conditions as *A. theophrasti* and *A. retroflexus*.

These results still need to be confirmed under field conditions but they reveal the different effect of phosphorus fertilization on the initial development of different weed species which can determine its competitiveness at initial crop development stages. Also, certain weed species can influence the efficiency of phosphorus use by crops, thus revealing a weed-crop interaction which can positively affect crop responses to phosphorus fertilizer supply.

# INFLUENCE OF COVER CROPS AND TILLAGE ON WEEDS IN SOYBEAN PRODUCTION IN SOUTHERN GERMANY

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Field experiments were conducted at the experimental station Lhinger Hof (71272 Renningen, Germany) and repeated at Kleinhohenheim (70599 Stuttgart, Germany) to investigate the possibility of no-tillage systems based on hardy cover crops to reduce the weed density in soybean in comparison to conventional tillage systems. Rye and barley were sown in autumn 2013 and in Mai 2014 (inflorescence emergence of the cover crop) seven different variants were performed. In the no-tillage variants the cover crops were treated by a crimper (CP), mulching machine (MU) or mowed by a cutter bar and removed by a loading wagon (MR) before sowing the soybean by a no-tillage machine. In the tillage variants, weeds were controlled by ploughing before soybean were sown. Afterwards weed control was carried out mechanically by hoeing (MH), except of the control variant (CO) where no weed control was executed. One further variant combined cultivation of cover crops with reduced tillage (RT). The tillage depth was 7 cm and soybean was sown by a drilling machine. To combat the weeds in the RT variant a hoeing machine was used. At the location Ihinger Hof an additional variant was investigated using strip-till-technique (ST) in combination with mulched cover crops and no-tillage sowing technique.

Emergence, biomass production and yield of the soybeans were determined in the different systems. Weed density as well as the species spectrum was investigated several times and the biomass production was evaluated. The experiments were conducted in a randomized complete split plot design with four replications. Data were analysis with the software R (Version 3.0.1.). Normality and homogeneity of variance were tested and the data subjected to an ANOVA. A linear mixed effected model was used with  $\alpha$ =5%.

Chenopodium album, Matricaria inodora and Polygonum convolvulus were the dominating weed species in the tillage variants. In variant CO up to 90 C. album plants m<sup>-2</sup> were counted, contrary to less than 5 plants m<sup>-2</sup> in the no-tillage systems CP, MU and MR. Sonchus arvensis and Stellaria media were primarily in the no-tillage systems CP, MU and MR. In the variant CO, where no weed control has taken place after ploughing, less than 10 S. media plants m<sup>-2</sup> were detected in contrast to 23 and 32 plants m<sup>-2</sup> in CP and MU. The weed control efficacy in the RT variant was higher than in the tillage treatment MH although same hoeing technique was used. The results have shown that weed suppression of the cover crop barley is better than of rye. The number of emerged soybean in the MU variant was reduced significantly about -50% compared with the mean of the other treatments cause of unfortunate seedbed conditions. Also CP had a negative effect on soybeans emerging at one location. In the ST variant there was a tendency, that weed control was affected positively from cover crop systems and soybean emergence from the tillage systems could be combined. The study has shown that cover crops in combination with no-tillage systems can reduce typical summer annual weeds especially C. album. Furthermore the results have shown that a combination of cover crop and reduced tillage (RT) can have a positive impact on the weed control in soybean. For a successful development of the soybean in the no-tillage systems the sowing technique is an essential factor. In due consideration of this fact, systems like CP, MR or ST could be a solution to replace chemical and mechanical weed control in local soybean production.

## INTEGRATED EFFECTS OF CROPPING SYSTEM AND HERBICIDES ON MAIZE COMPETITIVENESS

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The reduction of current yield losses caused by pests, pathogens and weeds are major challenges to agricultural production. Successful weed control in maize crop is characterized by implementation of different supportive and aimed measures. The system of measures is planed according to weed community composition and species abundance at certain agroecological conditions. The use of crop rotation is known to provide environmental benefits allowing a pests and weeds control and possible reduction in pesticide use. The most adopted crop rotation in Serbia is two crop rotation, maize-winter wheat, even though the recommendations are to include legume crops and conduct three crop rotation. Crop rotation also affected rotation of herbicides and their modes of action, which contributes to lowering of weed infestation. Weed competition affects physiological processes in maize plants and modifies their morphology. The aim of the research was to determine the advantages of double crop rotation in comparison to continuous maize growing in combination with different herbicide levels according to weed control effectiveness and crop morphological and physiological parameters which are important for maize competitiveness and productivity.

An experiment was set up in the field of the Maize Research Institute, Zemun Polje, in 2009. Experiment included two cropping systems: maize continuous cropping (MC) and maize - winter wheat rotation (MW). Both plots, continuous cropping and rotation, included subtreatments with different weed management: control without herbicide application; application of the herbicides isoxaflutole + acetochlor (750 + 768 g a.i.), which were used at the recommended dose (RD) and half of recommended dose (0.5RD). Each sub-treatment included four replications. After three years, the first comprehensive results of these cropping practices were obtained in 2011 and 2013. The experimental data of weed biomass (biomass of uprooted weeds) and maize harvest index, leaf area index (LAI), chlorophyll and carotenoids content were analyzed.

Weed biomass was significantly higher in 2013. It was decreased by herbicide application in both years and cropping systems, and there was no significant differences between RD and 0.5RD. Weed biomass was particularly lowered in RD treatment of maize-winter wheat rotation. With application of higher amount of herbicides, LAI was increased and harvest index decreased, mainly in maize-winter wheat rotation. Herbicide application also influenced chlorophyll and carotenoids content in maize leaf and they were higher compared to control in both cropping systems. LAI was significantly decreased (R2 = 0.826 in maize continuous cropping and R<sup>2</sup> = 0.640 in maize-winter wheat rotation) and harvest index was increased (R<sup>2</sup> = 0.482 and 0.122, respectively), parallel with weed biomass increase. In maize continuous cropping, chlorophyll and carotenoids content was significantly lowered parallel with weed biomass increase (R2 = 0.438 and R2 = 0.358, respectively) but in maize-winter wheat rotation only chlorophyll content was significantly lowered by weed competition.

According to obtained results, it could be concluded that competition pressure of weeds in rotation is weaker regarding morphological and physiological parameters of crop plants than in maize continuous cropping, and highly dependent on herbicide application.

#### EFFECT OF VARIOUS INNOVATIVE CROPPING SYSTEMS INCLUDING LEGUMES ON WEED COMMUNITY AND BIOMASS

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Intensive agricultural systems have often been based on optimizing the productivity of sole crops. These systems are based on low crop diversity and the use of large amounts of external inputs including fertilizers and pesticides. Such systems are widely criticized today for their negative environmental impacts. Increasing the use of herbicides in cropping system is also associated with increasing the occurrence of resistant and invasive weeds. As a result new sustainable agroecosystems are being sought which are based on an increase in spatial and temporal crop diversity. These systems often introduce legumes through intercropping and cover crops as living or dead mulch to add diversity to the main crop production. Nevertheless, few studies have quantified the effects of intercropping and cover crops on weed biomass and the weed community in relation to species traits (crops and weeds).

Field experiments were conducted in Angers, France from 2012 to 2014. The first experiment evaluated an annual intercropping of either frost sensitive faba bean or common vetch with oilseed rape on weed infestation compared with sole crops. In a second crop sequencing experiment, with a multi-year design, maize was sown in living cover crops of oats, alfalfa, English ryegrass, white clover and mixture of English ryegrass and white clover. A third experiment was carried out with the same cover crops species but with the cover crops destroyed at the maize sowing time. For all experiments weed population density, biomass and community diversity were measured at different stages. Shannon's and Simpson's diversity index were calculated to characterize treatment differences in plant community.

In all cases, intercropping resulted in lower weed biomass than sole cropping. In oilseed rapelegume intercrops; weed biomass was reduced compared to oilseed rape sole crop. A greater effect was observed with faba bean due to its faster crop growth compared to common vetch. In addition, we observed an interesting effect of intercropped systems on weed species. A greater number of weed species occurred in oilseed rape-faba bean intercrop and especially before winter.

In systems using maize with living and dead mulches, weed dry matter was lower in all treatments compared with conventional maize. Alfalfa and oats were most effective in weed suppression and displayed greater diversity and more uniform distribution of weed species comparing with other treatments. Each cover crop seems to modify its environment differently with consequences on weed community. Thus the English ryegrass had greater populations of *Convolvulus arvensis* L., whereas *Chenopodium album* L. is associated with alfalfa. This response may be due to the nitrophilic and morphological traits of these weeds species.

From the study of these various types of spatial diversification, we clearly show the interest of the addition of legumes to reduce weed biomass. Using intercrops or living and dead mulch tend to increase weed diversity and modify weed community in relation to crops and weed traits concerning their growth patterns and response to the environment.

# VICIA FABA FOR WEED MANAGEMENT: APPLICATION, EFFECTS AND MECHANISMS UNDERLYING WEED CONTROL

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Past years have seen increasing concerns about environmental impacts of some conventional farming practices, and this has raised the interest on implementing more sustainable agricultural practices as green manuring. Green manures can be powerful tools to increase crop yields and contribute to weed control. We have studied the use of the legume *Vicia faba* L. (faba bean) for weed control in maize. Previous *in vitro* experiments with *V. faba* aqueous extracts showed a great inhibitory activity on the most important common maize-related weeds in temperate areas (*Amaranthus retroflexus* L., *Echinochloa crus-galli* (L.) P. Beauv. and *Digitaria sanguinalis* (L.) Scop.) without damaging the crop (1). Greenhouse experiments of incorporation of *V. faba* into potted soil as green manure revealed a successful control of weed germination and early establishment immediately after incorporation into the soil, together with the enhancement of maize yields. Further field assays on weed-infested fields confirmed these results, but suggested a possible long-term limitation of these weed control effects, at least for infested fields.

The observed effects of *V. faba* green manure on weeds can be explained by both allelopathic and nutritional factors, due to an initial release of phytotoxic compounds from the decomposing plant material and a subsequent increasing availability of nutrients, especially N. We have found several monoterpenes in *Vicia faba* that can inhibit germination and early growth of weeds when applied as volatiles in the growing atmosphere or dissolved at low concentrations in aqueous solutions. These compounds may reach target weeds from the gaseous phase contained in soil pores or from the soil solution, being partially responsible of the global phytotoxicity observed in our assays.

Our results demonstrate that *V. faba* aerial biomass incorporated into the soil as green manure can contribute to early weed control in maize crops through several mechanisms. This ability for weed control, together with its additional agroecosystem services, makes *V. faba* a valuable tool to be included as a part of broader integrated weed management strategies in sustainable maize-based cropping systems in temperate regions.

1. Álvarez-Iglesias L et al. (2014) Allelopathy Journal 34: 299-314.
# THE CRITICAL PERIOD FOR WEED CONTROL IN SUGAR BEET (*BETA VULGARIS* L.) IN KAYSERI-TURKEY

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In Turkey, sugar is produced from sugar beets due to climatic conditions, as in most of Europe. Sugar beets prefer climates where the days are warm and the nights are cold. In this respect, sugar beets are grown mostly in the Central Anatolia Region. Sugar beet is grown throughout Turkey under irrigated conditions. The production is done under a contract of sugar companies. Weeds are a major constraint in sugar beet production. Understanding the critical period for weed control (CPWC) can be a tool for effective weed control and reducing the impacts of weeds.

In 2012 and 2013 field experiments were conducted during the seasonal growth periods in Kayseri, Turkey in order to assess the effects of 'Critical Period Weed Control' (CPWC) on sugar beet yield. A four parameter log-logistic model was adapted and used to assist in monitoring and analyzing the data. Data was obtained during periods of increased weed interference and as a comparison, during weed-free periods. The critical period for weed control in sugar beet based on a 2.5%, 5% and 10% acceptable yield loss level was calculated by fitting logistic and Gompertz equations to relative yield data. In 2012, the CPWC spanned from 177 to 489 GDD (Growing Degree Days) (17 to 41 Days After crop Emergence [DAE]) and in 2013 the CPWC ran between a period of 150 to 616 GDD (15 to 57 DAE) based on a 10% acceptable yield loss. Weed-free conditions needed to be arranged as early as a fortnight after crop emergence and maintained up to and including eight weeks thereafter to avoid more than a 10% loss in potato yield.

Acknowledgements: The author thanks the Research Fund of the Erciyes University for supporting project (Number FBA-12-3870).

# COVER CROPS FOR WEED SUPPRESSION IN PERSIMMON ORCHARDS IN TURKEY

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Agriculture is an important sector in Turkish economy and horticulture is a crucial component. Persimmon productions, mainly in the subtropical region in Turkey, have expanded rapidly in the past 10 years and exceed 20,000 tones. There are a lot of biotic and abiotic factors affecting yield in the persimmon orchards. Weeds are one of the most important yield limiting factors in the persimmon orchards. Alternative weed control methods are needed because of the various side effects of herbicides. Using cover crops for weed control in fruit orchards is one of the broadly applied alternative methods.

This study was conducted to determine weed suppressiveness of cover crops in persimmon orchards in the Kayseri in Turkey. *Trifolium repens* L., *Festuca rubra* subsp. *rubra* L., *Festuca arundinacea* Schreb., *Vicia villosa* Roth. and *Trifolium meneghinianum* Celm were used as cover crops in the experiment. Cover crop treatments were arranged in a randomized complete block design with four replications, and all cover crops were grown on the same plot during the experimental periods. Control plots such as weedy control, herbicide control and mechanical control were added as reference plots. In the second week of May (during the flowering periods of most cover crops), cover crop biomass and weed biomass were clipped from three 0.25 m<sup>2</sup> frames per plot and oven dried. Weed density and weed biomass were evaluated just before cutting the cover crops.

The lowest weed dry biomass was obtained from *F. rubra rubra* plots, and there were no significant differences among all other perennial cover crop treatments. Regarding the effect of cover crops on persimmon yields, the lowest yield was obtained from weedy control plots, while the highest yield was obtained from *V.villosa* plots. This research indicated that cover crops could be used as living mulch in integrated weed management programs to manage weeds in the hazelnut orchards.

Acknowledgements: The author thanks The Scientific & Technological Research Council of Turkey (TÜBİTAK) and Research Fund of the Erciyes University for supporting project (Project number: 1110647).

## CAN WEED MANAGEMENT IN VEGETABLE SYSTEMS BE IMPROVED BY COVER CROP SPECIES MIXTURES? STEP 1: SCREENING OF COVER CROP SPECIES AND VARIETIES

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Cover crops are an essential strategy in organic and low input arable and vegetable production, especially regarding weed reduction and N use efficiency, but their establishment and development can be unstable. A viable option to enhance and stabilise the agroecosystem services provided by cover crops is to sow mixtures of species having different functionality. Such mixtures have been reported to increase productivity, stability, resilience and resource use efficiency of cover crops mixtures. Therefore, our hypothesis is that weed reduction can also be improved by cover crops mixtures. However, it is not yet clear how diversity drives these mechanisms in arable and vegetable systems in Mediterranean climates.

Three experiments has been set, comprising (i) a growth chamber essay, including a germination test, (ii) a field experiment testing cover crops mixtures preceding an organic aubergine (*Solanum melongena* L.) crop, and (iii) a field catalogue. A total of 18 species are under study: 7 legumes, 6 *Brassicaceae* species, 4 *Graminaceae* and *Phacelia tanacetifolia* Benth (*Hydrophyllaceae*), represented by different cultivars, where available. In the present work only results from the first experiment will be shown.

To identify the species to include in the field experiment, cultivars of all 18 species were tested in the growth chamber essay. A RCB deign with 5 replicates was set. Phenology (BBCH scale) was measured three times a week. Above- and below-ground biomass, plant height and root length were measured 26 days after sowing. A Wilcoxon rank sum test was performed to compare cultivars performances. The orthogonal contrasts method was utilized to compare performances of species within families.

Significant differences were found between and within species, mainly in terms of above-ground biomass. Based on the results, we consider that functional groups should correspond to the botanical family. The family *Leguminosae* was split in two functional groups: large-seeded legumes, with faster development and higher biomass production, and small-seeded legumes. Therefore, eight candidate species and their relative cultivars were identified for the field experiment, based on higher biomass production, plant height, and extension of the root system as traits linked to good competitive ability. Species and cultivars not included in the field experiment will be grown in the field catalogue.

The field experiment will be arranged in a RCB with 3 replicates and 18 treatments. To test the relative importance of functional composition and functional diversity in improving and stabilising the agroecosystem services expected from cover crops, we designed 4 mixtures of 2, 4 mixtures of 4 and a mixture of 8 species to create a gradient of diversity: (i) pure stands; (ii) co-presence of 2 functional groups; (iii) diversity within 2 co-occurring functional groups, (iv) co-presence of 4 functional groups, (v) diversity within 4 co-occurring functional groups.

# IMPACT OF COVER CROPS AND CROP ROTATIONS ON THE RE-ESTABLISHMENT OF THREATENED ARABLE PLANTS

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Due to intensification of land use the arable flora belongs to the groups of plants with the highest percentages of threatened species in Europe. As these species significantly contribute to the functional diversity of agro-ecosystems, their conservation implies an important challenge for biodiversity management in arable landscapes. A problem with their conservation is that these plants frequently grow where they are endangered by intensive cultivation. Therefore, the objective of the project 're-introduction of rare arable plants on organic farms in Germany' is to develop suitable methods to successfully re-establish threatened species on fields with favorable management conditions.

In a corresponding field experiment on calcareous soils in the Munich Plain (Germany) we tested the effects of different crop rotations, tillage treatments, sowing dates and sowing densities on the establishment of three endangered species (*Legousia speculum-veneris* (L.) Chaix, *Consolida regalis* Gray, *Lithospermum arvense* L.) which are mentioned on the list of threatened European arable plants by Storkey et al. (2012). The results presented here demonstrate how different crop rotations affect the establishment of the rare plants.

In autumn 2011, a mixture of 500 seeds/m<sup>2</sup> of *L. speculum-veneris*, 200 seeds/m<sup>2</sup> of *C. regalis* and 150 seeds/m<sup>2</sup> of *L. arvense* were sown to each of the following crop rotations (five repetitions each):

-  $1^{st}$  yr no crop –  $2^{nd}$  yr rye (350 seeds/<sup>2</sup>) –  $3^{rd}$  yr rye (350 seeds/m<sup>2</sup>)

-  $1^{st}$  yr spelt (40 husked seeds/m<sup>2</sup>) –  $2^{nd}$  yr rye (350 seeds/m<sup>2</sup>) –  $3^{rd}$  yr rye (350 seeds/m<sup>2</sup>)

-  $1^{st}$  yr spelt (160 husked seeds/m<sup>2</sup>) –  $2^{nd}$  yr rye (350 seeds/m<sup>2</sup>) –  $3^{rd}$  yr rye (350 seeds/m<sup>2</sup>)

-  $1^{st}$  year rye (350 seeds/m<sup>2</sup>) –  $2^{nd}$  year grass clover (3 g/m<sup>2</sup>) –  $3^{rd}$  yr rye (350 seeds/m<sup>2</sup>)

These crop rotations represent a gradient of deteriorating establishment conditions for the target species. Field records were conducted during the 3rd year and soil seed bank samples were collected two years after initial sowing.

Both analyses indicate that successful establishment mainly depends on crop competition during the 1<sup>st</sup> year. Thus, the soil seed bank of all three target species exceeded the initially sown seed numbers when they were disseminated without cover crops in the 1<sup>st</sup> year. However, the experiments also showed varying sensitivity to crop competition among the target species. While 58% of the seeds of *L. speculum-veneris* survived the cultivation of rye and grass clover (treatment with the highest crop competition), *C. regalis* and *L. arvense* had almost disappeared from the soil seed bank of these plots.

We conclude that re-establishment on fields with favorable management conditions can provide an efficient tool to conserve threatened plants in agricultural landscapes. However, successful conservation may also necessitate a spatially differentiated concept where management is adapted to the requirements of individual species.

# EFFECTS OF FLOODING AND FARMING PRACTICES ON OF AMPHIBIOUS PLANT POPULATIONS IN TEMPORARILY FLOODED ARABLE FIELDS

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During the past century the arable biodiversity experienced a severe decline due to an intensified land use and habitat melioration leaving poorly structured organism communities with a few common species. Therefore, programs with the objective to conserve the arable vegetation were implemented since the 80ies. However, most of these programs did not focus on the vegetation of temporarily flooded fields which can harbour a specialized flora with various rare species, some of which are already threatened by extinction.

In order to provide feasible recommendations to preserve these communities we studied the impact of farming practices and hydrological regimes on their populations. Therefore, we tested the effects of different management measures (soil tillage, competition by crops, fertilization and herbicide application) and flooding on populations of three model species (*Limosella aquatica* L., *Myosurus minimus* L., *Peplis portula* L.) in a field experiment.

The study was carried out 2012– 2014 in seven regularly flooded field depressions on a conventionally managed farm in NE Brandenburg. In 2012 178 plots were established where at least one of the three target species was present and the initial population density was recorded. In autumn all plots were ploughed in order to equalize the starting conditions. Combinations with and without crops, fertilization and herbicide applications, respectively, were randomly assigned to the plots. Treatments were carried out manually. The water level was assessed several times each year and classified to one of the categories moist, water-logged or flooded.

Analyses indicate that flooding promotes the establishment of *L. aquatica* and *P. portula*. These species were not able to establish under competition of terrestrial arable plants and crops. The effects of farming practices depend on the timing of seedling emergence. While populations of winter-annual species like *M. minimus* were severely reduced by herbicide applications and promoted by soil tillage, summer-annuals like *L. aquatica and P. portula* were not affected by these treatments. Fertilization increases the size of inflorescences (*M. minimus*) or the whole plant (*L. aquatica, P. portula*). Competition by crops seemed to support *M. minimus* and to suppress *L. aquatica and P. portula*.

We conclude that both cultivation practices and the water regime are relevant for the conservation of amphibious plant species in arable fields. Although many of these rare species scarcely suffer from herbicide treatments due to germination at higher temperatures in early summer, at least sites with significant populations of cold germinators like *M. minimus* still require a limitation of weed control. For conservation of the threatened summer annuals maintaining a regular flooding regime appears more important.

# EFFECT OF CROP VARIETY AND ORGANIC MULCHES ON WEEDS IN POTATO

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Potato is an annual crop which plays an important role in human's source of food. Weeds remain one of the most significant agronomic problems associated with the production of organic crops, decreasing the quality and quantity of potato tubers. Mulching is a weed control method used in agriculture throughout the world. Organic mulches are more popular in cropping systems. Mulching also decreases an annual weed infestation due a sufficient layer of mulch can inhibit weeds emergence. The aim of this study was to determine the effect of 2 potato varieties in combination with different organic mulches on weeds.

The field experiments were carried out in 2013–2014 at the Elmininkai Experimental Station, Lithuanian Research Centre for Agriculture and Forestry. Early maturing potato variety 'Solist' and later maturing potato variety 'Mocart' were growing. Spreading of wheat straw and peat on potato fields surface shortly after crop planting (mulching) and after hoeing and cultivations (two times) were compared with non-mulching but mechanically weeded control (hoeing and cultivation twice before and twice after potato germination). The experimental design was a split plot with four replications. Chopped straw and peat was applied by hand in 10 cm thick layer on soil surface. Population of weeds was measured using 0.5 m x 0.5 m quadrates; four for each subplot. All weeds were calculated and collected from a countable area and weighed to determine the weed dry matter. The number of weeds was recorded at the potato germination growth stage and later on at a ten day intervals until canopy closure. Dry mass of weeds was recorded during the final assessment.

The main weed species were *Fallopia convolvulus* (L.) Löve, *Viola arvensis* Murray, *Chenopodium album* L., *Galium aparine* L., *Echinochloa crus-galli* (L.) P. Beauv. and *Poa annua* L. Weed species were affected by organic mulches. Earlier in the season (after crop germination) and until canopy closure mulching reduced the number of *F. convolvulus*, *G. aparine* and *C. album*. This reduction was significant only in the treatment with wheat straw mulching. Only late assessment revealed significant differences in the number of *V. arvensis*, *E. crus-galli* and *P. annua*. The number of *F. convolvulus* and *G. aparine* was significantly lowest in the wheat straw mulched plots than with peat at the final assessment. The effect of both organic mulches was similar on *C. album*, *V. arvensis*, *E. crus-galli* and *P. annua*. Crop variety similarly affected the number of weeds, except *C. album* and *E. crus-galli*. Later maturing potato variety 'Mocart' better suppressed *C. album* and *E. crus-galli*. There were no statistically significant differences.

Significantly highest total weed dry mass was recorded in the treatment with mechanical weed control (267 g m<sup>-2</sup>). Organic mulches without hoeing and cultivation produced the least control as compared to organic mulches with hoeing and cultivation but the differences were no statistically significant. The highest dry mass of weed was observed in the treatments with peat and the lowest in the treatments with wheat straw for both using methods. Significant statistical differences were observed between peat and wheat straw. The response of weeds to crop variety was also observed for total weed dry mass, where the highest weed mass (18 %) was recorded in the early maturing potato variety 'Solist'. Their differences were not statistically significant. Crop variety had no influence on weeds mass.

# EFFECTS OF RICE STRAW AND DIGESTATE SOIL INCORPORATION ON GROWTH OF WEEDY RICE (*ORYZA SATIVA*) AND BARNYARDGRASS (*ECHINOCHLOA CRUS-GALLI*)

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The recent development of the bioenergy sector has made significant amounts of digestate available, as by-product of anaerobic digestion of biomasses. As several plants have been set up in the Italian rice area, there is a growing interest on using digestate as organic fertilizer for rice cultivation. In this crop, straw is mainly incorporated into the soil, being the main organic input. Alternatively, straw can be removed from the field or burned.

The study, conducted during 2014 in Italy, aimed at comparing the effect of two straw management practices (presence or absence of rice straw incorporation) and four different fertilizations (no fertilization, whole digestate, solid digestate, and urea) on the growth of two rice weeds: weedy rice (*Oryza sativa* L.) and barnyardgrass (*Echinochloa crus-galli* P. Beauv.). The study was carried out in 15-L plastic pots (with three replications) filled with paddy soil. Before pot filling, the soil was sieved to remove most of the straw residues already present. For the treatments that included straw incorporation, an amount of rice straw equivalent to 7 t ha<sup>-1</sup> was added to each pot and mixed with the soil. Digestate or solid digestate was added at a rate of 22 t ha<sup>-1</sup> and incorporated in the first centimeters. The pots that received urea were fertilized at a rate equivalent to 100 kg N ha<sup>-1</sup>. Twenty seeds of *O. sativa* or *E. crus-galli* were seeded on the soil surface of each pot; during plant growth two additional top-dress fertilizations with urea were given to all the pots at a rate equivalent to 30 kg N ha<sup>-1</sup>. Pots were maintained in open field and kept flooded with 3 dry periods of few days. Several times during the season, different growth parameters were assessed: plant height, growth stage, tiller number, flag leaf length, and leaf chlorophyll content (SPAD readings). At harvest, plant biomass, number of culms and panicles and seed biomass were determined.

At the last assessment, for both weedy rice (*O. sativa*) and *E. crus-galli*, no significant effects of straw incorporation and fertilization were found on plant height, SPAD readings, culm and panicle number. In weedy rice, plant biomass varied according to the fertilization adopted but only in absence of straw incorporation, with the unfertilized pots showing the lowest plant weight (3.6 g plant<sup>-1</sup>), followed by whole digestate. The highest weight was recorded by both solid digestate and urea with 5 g plant<sup>-1</sup>. *E. crus-galli* showed in absence of straw incorporation, the lowest plant biomass in the unfertilized pots (2.7 g plant<sup>-1</sup>) and the highest in those with urea (5.0 g plant<sup>-1</sup>). Pots with straw incorporation showed differences in plant biomass only between urea (6.4 g plant<sup>-1</sup>) and all the other treatments (3.5 g plant<sup>-1</sup>). Seed biomass varied among fertilization in *E. crus-galli* only, showing the highest values in pots fertilized with urea. Differences in terms of weed growth were more evident without straw incorporation. Urea determined the highest growth values while digestate application showed intermediate values between mineral and non fertilization.

# COMPARISON AMONG DIFFERENT AVAILABLE STRATEGIES FOR WEED CONTROL IN MAIZE

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Weed competition can cause severe yield reduction in maize cultivation. Since the sixties the large availability of different herbicides has allowed maize growers to reach appreciable increase in the yield per hectare. At present different strategies based both on chemical and mechanical control can be adopted to control weed infestation. The aim of the study was to compare different weed control strategies currently available in terms of type (chemical, mechanical or a combination of both) that fulfill the requirements of the Directive 2009/128/EC and the guidelines contained in the National Action Plans adopted by the European member states.

The study was conducted in 2014 at Turano Lodigiano, Lombardy region, North-west of Italy on a field of about 6000 m<sup>2</sup>, with 90 plots of 28 m<sup>2</sup> each. Plots were completely randomized with three replications for each strategy and several untreated plots. Five weed control strategies were compared: chemical pre-emergence (PRE), chemical post-emergence (POST), chemical pre+post emergence (PRE+POST), mechanical control (MECH), and chemical and mechanical control (CHEM+MEC). In particular, nine different mixtures of herbicides were applied in PRE, 5 mixtures in PRE+POST and 5 in POST. In MECH, weed control was based on a combination of spring-tooth harrowing and ridging, in CHEM+MECH, 4 herbicide applications were combined with ridging. The effects on weed infestation were assessed on key weeds (*Abuthilon theophrasti, Amaranthus retroflexus, Chenopodium album, Echinochloa crus-galli, Panicum dichotomiflorum, Poa annua, Portulaca oleracea, Setaria viridis* and Solanum nigrum) by measuring plant density (plants/m<sup>2</sup>), ground cover (%) and by visually evaluating overall efficacy on a percentage base. Crop yield was also determined for all tested treatments.

Untreated plots showed a severe and homogeneous infestation (97.56 plants/m<sup>2</sup>). Highest weed plants/m<sup>2</sup>), densities were assessed in MECH (36.00 POST (29.33 plants/m<sup>2</sup>), PRE (16.07 plants/m<sup>2</sup>) and CHEM+MECH (9.89 plants/m<sup>2</sup>). Maximum weed cover values were observed in MECH (46.67%), POST (34.38%) and PRE (9.66%). The visual efficacy of weed control in the different strategies compared was generally close to 100%. A negligible lack of efficacy was observed in PRE strategies for the formulation containing isoxaflutole (50 g L<sup>-1</sup>) and thiencarbazone-methyl (20 g L<sup>-1</sup>) at 2 L/ha field rate and in POST for the formulation containing terbuthylazine (16.94 g L<sup>-1</sup>), S-metolachlor (28.23 g L<sup>-1</sup>) and mesotrione (3.39 g L<sup>-1</sup>) at 4 L/ha. The grain yield was remarkably high (18.5 t/ha on average) without significant differences between strategies, with the exception of untreated plots (0.53 t/ha) and MECH (3.67 t/ha).

Overall, the chemical control showed a high efficacy towards most of the weeds. The lack of efficacy seen for MECH strategies was mainly due to the incomplete action of the spring-tooth harrowing intervention.

# ARE WHEAT HYBRIDS MORE AFFECTED BY WEEDS THAN CONVENTIONAL VARIETIES?

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Wheat hybrids have been cultivated in Europe on 250.000 ha in 2013. Hybrids are commonly seeded at much lower seeding rate compared to the common varieties; the low seeding rates may results in lower competitive ability of hybrids toward weeds, especially during first growth stages of the crops.

A field-experiment was conducted to compare weed density and yields performance of conventional varieties and hybrids. The study was carried out during the campaign 2013-2014 in Piemonte, north west of Italy, at three sites (Borgo d'Ale, Moncrivello and Grugliasco) characterized by different soils and weed infestation conditions. The varieties compared were the conventional Illico, and the hybrids Hystar and Hyxo, seeded at 220 kg/ha, 75 kg/ha and 60 kg/ha, respectively. Four plots (7m<sup>2</sup> each), covered by plastic films during herbicide spraying, were randomly selected in contiguous fields, characterized by an homogenous infestation, cultivated with the three varieties indicated above. In each plot weed density (plants/m<sup>2</sup>) and weed cover on the ground (%) were evaluated. Weed assessments, three reps per plot, were performed two times during the growing season (at early tillering stage and at the beginning of stem elongation).

Overall, the highest weed densities were generally encountered in the fields cultivated with hybrid varieties, with Hystar in particular. At Borgo d'Ale the most abundant weeds were Stellaria media, Matricaria chamomilla, Panicum dichotomiflorum and Viola tricolor, at Moncrivello Stellaria media and Veronica persica; at Grugliasco Poa spp., Stellaria media, Veronica persica and Ranunculus arvensis. Overall, at the first assessment, no significant differences were found between varieties. Differences between the conventional varieties and hybrids were larger at the second assessment, when climatic conditions were more favorable to weed growth. Weed infestation was rather diversified at the three sites. At Borgo d'Ale at the second assessment, the weed density was 198 plants/m<sup>2</sup>, 192 plants/m<sup>2</sup> and 128 plants/m<sup>2</sup>, in Hystar, Hyxo and Illico cultivars, respectively. At Moncrivello, during the same assessment, the highest weed density was 242 plants/m<sup>2</sup> in Hystar plots, while nonsignificant differences were noted among Hyxo (172 plants/m<sup>2</sup>) plants/m<sup>2</sup> and Illico (161 plants/m<sup>2</sup>). At Grugliasco the weed infestation was lower compared to the other sites: 56 plants/m<sup>2</sup> in Hystar and Hyxo plots, and 60.8 plants/m<sup>2</sup> in Illico, without significant differences. Similar results were obtained in terms of weed cover with the highest values (between 40% and 60% at the second assessment), observed in hybrid plots. The yields were not significantly affected by the varieties, regardless the site location.

In conclusion, the degree of weed infestation in hybrids was in general higher with hybrid varieties than in conventional varieties. However, the high tillering ability of hybrids allows them to contain efficiently weed competition, without significant impact on yields.

# WHEAT CULTIVARS VERSUS ALOPECURUS MYOSUROIDES (L.) – WHAT MAKES A WINNING CULTIVAR?

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It has long been appreciated that cultivars of wheat vary in their ability to compete with weeds for resources. However, competitive cultivars are rarely a component of integrated weed management strategies. This is owing to various factors, such as limited information on the competitive strength of cultivars and how to best integrate them into weed control strategies. With herbicide resistance becoming widespread and a dwindling pool of herbicides to utilise, interest is returning to cultural control methods. A quick and non-destructive method of grading cultivars for their competitive ability is required. In combination with advice on their utilisation, competitive cultivars may become a valuable addition to weed management strategies.

In this study, 8 wheat cultivars were assessed for their ability to suppress the growth of *Alopecurus myosuroides* (L.), a problematic weed species in the UK. The cultivars were grown with *A. myosuroides* in outdoor containers over three. The container experiment was arranged as a randomised complete block design with three replicates, and throughout growth various wheat traits were measured throughout the growing season. The aim was to establish what traits were related to the ability of a cultivars to reduce the fitness of this weed species, and if traits can be used to predict the competitive ability of wheat cultivars. Statistical analysis includes the use of principal component analysis and linear mixed models. Plant height was examined via an unconstrained generalised logistic curve model and stem production explored using a split-line model.

Many wheat traits appear to contribute to a cultivars ability to suppress *A. myosuroides*. These include early height, the size of early leaves and traits related to time to maturity (spike emergence and time to maximum stem number). These traits were components of a PC, implying the importance of a rapid rate of development in the ability to suppress *A. myosuroides*. However, these traits differ in their importance in competitive outcomes over the three years of study. This strengthens the case for a suite of traits to be identified and used in the assessment of wheat cultivar suppressive ability.

# EFFECTS OF COMBINATION OF DIFFERENTS AGRONOMIC PRACTICES ON WEED IN FRENCH CEREALS ROTATION

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Weed control based solely on herbicides becomes more and more difficult in French cereals, rapeseed and pulses. In this context, other agronomic practices become essential to manage weed population. The effect of each agronomic practice is well known, but references are missing on the effect of combinations of these practices at short and long-term weed control and on the risk of emergence of resistance. The aim of the study was to analyse the effects of different cropping systems on the flora.

This paper presents the results obtained in a trial carried out from 2006 to 2014 by ARVALIS Institut du vegetal at Epieds, near Evreux (27) on a shallow stony silt soil, where, since 1996, the field is divided in two parts. One part is ploughed every year and on the other part, the soil is never ploughed. Crop rotation, date of sowing (only for cereals) and stubble management are the main experimental factors crossed. They vary as shown below:

1: stubble tillage, rapeseed/wheat/pulses/wheat, cover crop before pulses, late sowing date for wheat (beginning of November).

2: no difference with 1, except early sowing date (beginning of October)

3: no stubble tillage or cover crop during the intercropping period, rapeseed/wheat/wheat, early sowing date

4: stubble tillage, continuous wheat, late sowing date

These 4 systems are studied with and without ploughing. Each of the eight plots (24mx60m) presents an untreated zone (10mx24m) which is chopped before seedling of weed. These untreated zones allow to assess the impact of the agronomic practices in absence of herbicides. On the rest of each plots, herbicides are carefully selected and applied in order to maximise efficacy. 26 species of weed were present in the untreated zone at the beginning of the trial. *Poa annua, Lolium multiflorum, Alopecurus myosuroides, Galium aparine, and Stellaria media* were the most frequent.

After 6 years of experimentation, we notice that:

• Delaying sowing date (1 versus 2) of winter wheat allows to include an additional stale seedbed if weather-permitting.

• In continuous wheat (4) intercropping period is longer and sometimes allows to do 1 or 2 supplementary stubble digging.

• Based on the observations on the untreated areas, ploughing is the most impacting practice on weed's density whatever the rotation or sowing date is. The impact of ploughing is stronger in cases of early sowing and when other practices (long rotation, shallow cultivation) are absent.

• On winter wheat, comparisons between 1 and 4 shows a very important effect of the crop rotation in non-ploughing systems. Diversity and density of weeds decrease in treated and untreated areas according to the rotation. In non-ploughing system, in treated area and in all conditions, density of weeds is higher compared to the density in the ploughing system. Grass weeds are very frequent and herbicides efficacy is not perfect in spite of autumn followed by spring applications. In 2014, in winter wheat, foliar herbicide fail to control blackgrass and rye grass in continuous wheat, short rotation (3) and condition 2; sign of a shift of foliar herbicides efficiency.

# OILSEED RAPE CROP DENSITY AND DEMOGRAPHIC TRAITS OF THREE ANNUAL WEED SPECIES

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As a result of a rising demand for renewable energy sources in past decades the acreage for energy crops has been increased more than tenfold. In Central and North-West Europe this also resulted in an expansion of oilseed rape cropping area. In Germany *Anchusa* sp., *Geranium* sp. and *Viola* sp. are three of the most important weeds in oilseed rape. Therefore, we investigate how crop density of oilseed rape affects demographic traits of *A. arvensis* (L.) M. Bieb, *G. pusillum* (L.) and *V. arvensis* Murray.

A semi field trial was set up in the year 2013 at the research area of the Crop Health Department from the University of Rostock (N54° 4.07573, E12° 4.9365; Mecklenburg-Western Pomerania). Smallest experimental units were pots digged in soil, i.e. large black plastic tubes (pot: A = 0.6 m<sup>2</sup>, V = 285 I) with holes in the bottom. These pots were established in the year 1998 and from that moment continuously managed as "mini" arable fields (rotations of oil seed rape and winter cereals). The experimental design was a randomized block design with four repetitions, whereby each block contained 12 pots. In the year 2013 average annual temperature and annual precipitation in Mecklenburg-Western Pomerania were 8.9 °C and 607.8 mm, respectively. Furthermore, the region is characterized by an early summer drought season. Four densities of oilseed rape (0, 10, 50 and 90 plants per m<sup>2</sup>) were cultivated in combination with A. arvensis, G. pusillum or V. arvensis. Each weed species was randomly sown into pots (850 weed seeds). The crop was cultivated in a fixed sowing scheme according to target crop density, i.e. constant row spacing (15 cm) and varying plant numbers in row. The crop growing season run from September 4<sup>th</sup> (2013) to July 1<sup>st</sup> (2014). During this period weed population density and phenology was pursued by counting weed plants at six dates. At each date the entire weed population was subdivided into three separate development stages (seedling, juvenile and adult) and counted. To evaluate the effect of crop density on demographic traits of A. arvensis, G. pusillum and V. arvensis, densities of the phenological stages at several times of cropping season were analyzed and tested for differences using Kruskal-Wallis Test (R Core Team, 2013; package agricolae).

First results have shown no significant differences in seedling densities of *A. arvensis*  $(0 - 17 \text{ plants per m}^2)$ , *G. pusillum*  $(182 - 572 \text{ plants per m}^2)$  and *V. arvensis*  $(2 - 13 \text{ plants per m}^2)$  between varieties of crop density (P > 0.05). However, the density of *A. arvensis* at the end of the cropping season ranged from 10 to 80 plants per m<sup>2</sup>, whereas the total number of *A. arvensis* was significantly decreased by crop density (P = 0.03). In spite of the fact that abundance of *G. pusillum* and *V. arvensis* respectively extended from 115 to 257 and 0 to 12 plants per m<sup>2</sup>, no significant differences in total number of weeds between crop densities (P > 0.05) at the end of cropping period could be found.

# MODELING POPULATION DYNAMICS OF *BROMUS JAPONICUS* IN WHEAT IN ARID ENVIRONMENTS: SIMULATING VARIOUS SCENARIOS OF WEED MANAGEMENT

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*Bromus japonicus* is a problematic species in wheat fields of southeastern Iran causing about 25% yield loss in winter cereals. Although cultural methods such as tillage, crop rotations, and crop density are shown to be effective for the control of *B. japonicas*, the current applied methods are mostly restricted to herbicide application. A population dynamic model was developed in order to evaluate the potential strategies to achieve an integrated decision making process that may decrease the risks threatening our agro-ecosystem.

Field experiments were conducted for model parameterization. The current study model was constructed based on biological behavior of *B. japonicus* influenced by environment and management practices. The initial weed seed bank of *B. japonicus* was estimated at 7643 seed m<sup>-2</sup> with a standard error of 1031. The model simulates the infestation levels of *B. japonicus* as affected by different scenarios of wheat cultivar, wheat planting density, herbicide dose, and tillage types over the future ten years.

For more competitive cultivars, a high planting density could maintain the seed bank population constant over ten years. Some modifications in other mechanical or cultural methods aid reducing herbicide application rate. If the wheat density increases to 500 plants m<sup>-2</sup> no difference can be found between reduced and recommended herbicide dose rate. Both the shallow and deep tillage give a satisfactory control of *B. japonicus*, and if these are combined with a reduced herbicide rate, a significant decrease in the *B. japonicus* soil seed bank can be obtained. An integration of high planting density of wheat as a low cost alternative with reduced dose of an effective herbicide with preceding shallow tillage practices could reduce the population to levels below the problematic threshold. Furthermore, the method is consistent enough to prevent further distribution of *B. japonicas* population.

Keywords: Population dynamics, *Bromus japonicus*, cultural practice, herbicide dose, integrated management.

# MEDICINAL AND AROMATIC PLANTS TOLERANCE TO DIFFERENT WEED CONTROL METHODS

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Medicinal and Aromatic plants (MAPs) characterize the typical flora of the countries around the Mediterranean basin. Particularly, the species *Origanum vulgare* spp. *hirtum* L. Greek oregano and *Salvia officinalis* L. sage are the most common in the phytogeographical territory of Greece. Weeds constitute the major problem of the cultivation especially during the first years of MAPs establishment. The purpose of this experiment is to examine the tolerance of the studied MAPs to the application of several weed control methods, investigating their impact on productive capacity and their essential oil chemotype composition.

The experiment was established in Arginio (Western Greece) the year 2014. The MAPs were planted in plots of 10,125 m<sup>2</sup> with three replicates in a fully randomized experimental design. The treatments consist the following eight different weed control methods, with weed control and weed free control to be included; Pre1: pre-emergence application with mixture of metribuzin and pendimethalin at rates 140 g a.i. ha<sup>-1</sup> and 136,5 g a.i. ha<sup>-1</sup> respectively, Pre2: mixture of metribuzin and pendimethalin at rates 210 g a.i. ha<sup>-1</sup> and 91 g a.i. ha<sup>-1</sup> respectively, Post: post-emergent mixture of the herbicides cycloxydim and metribuzin 0.3 L a.i. ha<sup>1</sup> and 175 g a.i. ha<sup>1</sup> respectively, R-0.7: glyphosate (0,252 L a.i. ha<sup>-1</sup>) and R-1.5: (0.54 L a.i. ha<sup>-1</sup>), FI: flaming propane at rates 99 kg ha<sup>-1</sup>, 119 kg ha<sup>-1</sup> and 137 kg ha<sup>-1</sup>. The effect of flaming was evaluated taking measurements on weeds and MAPs at 1, 7, 14, 21 and 28 days after treatment. The post-emergence applications with herbicides were carried out when the plants average height was 20 cm at their early vegetative stage. The dominant weeds in the experimental field were Cyperus rotundus L. and Sorgum halepense Pers. and in lower populations the weeds species Chrozophora finctoria L., Amaranthus viridis L existed. In order to estimate the crops growth the following measurements were taken every 15 days from three plants per plot: plant height, number of shoots and number of leaves in three shoots. Furthermore, fresh and dry matter was estimated after oregano harvest at fully anthesis and sage harvest at the late vegetative stage. The efficacy of weed control methods on weed population was estimated taking measures on weed density by 50 by 50 cm quadrat and on the fresh and dry weights of the weeds.

Based on visual measurements, sage plants showed higher tolerance to all the herbicide treatments compared to oregano plants. Similarly, the fresh and dry matter of sage plants was higher compared to oregano plants. Particularly, the application of post-emergent herbicide with the mixture cycloxydim and metribuzin had no significant effect on MAPs yield with the values of sage and oregano fresh matter do not differ significantly from those derived from the weed free control treatment. Concerning the flaming treatment the studied MAPs showed a tolerance to the application of 99 kg ha<sup>-1</sup> propane. The essential oil yields and their chemical composition were influenced by the different weed control treatment.

#### WEED POPULATION DYNAMICS UNDER SITE-SPECIFIC WEED MANAGEMENT

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Herbicide consumption could be decreased by adoption of site-specific weed management (SSWM). This method enables the application of herbicides in areas where the weed abundance exceeds a damage threshold. Accurate threshold values are, however, needed for reliable use of SSWM. This paper evaluates the effect of SSWM on weed populations.

SSWM was used on a 3.07 ha experimental field during 2011–2014 in a rotation of winter wheat and winter rape. The experimental area was split into cells of 6 × 10 m. A total of 512 cells were arranged into 16 blocks, which allowed the randomization of four treatments in four replications. Treatment 1 represented blanket spraying regardless of weed infestation whereas the treatments 2 - 4 consisted of SSWM with increasing thresholds used for individual weed groups. Weed infestation was evaluated in spring of each year prior to post-emergence herbicide application. The density of each weed species was evaluated manually by counting individual weeds in four samples taken in the central part of each cell. Treatment maps were created for each weed group based on weed abundance data and treatment thresholds. Following thresholds were used in treatments 2; 3 and 4 respectively: 0.2; 0.5 and 1 plant m<sup>-2</sup> for *Galium aparine* L. and *Cirsium arvense* (L.) Scop., 5; 10 and 15 plants m<sup>-2</sup> for *Tripleurospermum inodorum* (L.) Schultz-Bip. and for annual grasses. Thresholds of 10; 20 and 30 plants m<sup>-2</sup> were used for other dicotyledonous weeds. Herbicide application against individual weed groups was performed separately using a sprayer equipped with GPS and boom section control. Differences in population density among treatments were analysed by ANOVA.

SSWM led to significantly increased density of *G. aparine* in 2013 and 2014, and its abundance increased with rising threshold value. Significant differences were found between treatments 1 and 4 in 2013 (p = 0.076) and 2014 (p = 0.007). The overall increase in *G. aparine* density cannot be attributed to the SSWM only, because high densities were also found with the blanket treatment. The causes of this increase can be found in limited crop rotation of winter crops combined with minimum tillage practices. Similarly, density of the *T. inodorum* population increased substantially on SSWM plots in the third and fourth experimental years. Treatment 1 was found to be significantly different from treatments 3 and 4 in 2013 (p = 0.053 and p = 0.034, respectively) and from treatment 4 in 2014 (p = 0.075). Annual monocotyledonous weeds were represented mostly by *Apera spica-venti* (L.) P.B. Plant densities of this group were also higher SSWM and increased slightly with increasing threshold value, but the herbicide savings were acceptable here even in last experimental year. The effect of SSWM was insignificant for other weed groups. Based on these results, very low SSWM thresholds are recommended for *G. aparine* and *T. inodorum*. Higher threshold are appropriate for *A. spica-venti* because of absence of a long-term soil seed bank.

## SYNERGY AMONG CULTURAL WEED CONTROL MEASURES

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Weeds are one of the most important biological production constraints in a wide variety of crops and need attention. In most production systems, the focus is on herbicidal control, as herbicides are effective and comparatively cheap. The strategy allows farmers to focus on the short-term: avoidance of yield reduction in the current crop. Due to the gradually decreasing number of available herbicides, the sustainability of herbicidal control is seriously at stake. Relying on fewer herbicides increases the likelihood that herbicide resistant populations will develop, thereby undermining the durability of this strategy. The challenge is to broaden the foundation underlying current weed management. Augmenting direct weed control with cultural control measures seems a promising route to more sustainable weed population management.

In a weed population management strategy, focus is not just on direct control of weed seedlings. The life cycle of weeds offers many more options to interfere and these interventions can be brought about through modifications in cropping practices, like for example the use of more competitive cultivars, transplanting, or the application of a false seed bed technique. The intention of all measures is to lower the weed seed bank density (representing the potential weed problem), to lower the weed plant density (the plants that will eventually compete with the crop) and/or to lower the impact of those weed plants on crop production (competitive ability). It is well established that the strength typically has to come from a combination of measures, as individual cultural control measures are not as effective as herbicidal control. It is therefore important to establish whether synergy can be generated by combining cultural control measures that target different phases of the weed's life cycle.

For addressing this question a life cycle stage weed population model was developed. The model was used to determine equilibrium weed seed densities following individual and combinations of cultural control measures. The equation to express the equilibrium weed seed density reinforces that the life cycle of a weed consists of two major flows. The first flow represents the plant phase and more in particular the development of a weed seedling to a seed producing plant. The second flow represents the passage of newly produced seeds to their settlement in the weed seed bank. Both flows are connected through two important transitions. The first transition from plant to seed is characterized by multiplication, as each weed plant is able to produce multiple seeds. The second transition from seed to plant represents stock formation, and reflects the residence time of the seeds in the weed seed bank. Both flows as well as transitions can be influenced through cultural control measures and be used to lower the weed seed and weed plant density.

It is shown that an analysis of cultural control measures expressed in their influences on the discerned flows and transitions resolves whether synergy between measures is to be expected. This insight is essential for selecting the best possible combination of cultural control measures for development of sustainable integrated weed management strategies.

## WEED MANAGEMENT STRATEGIES FOR POPLAR ENERGY CROPS

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Crops intended for biomass production to be used as fuel in energy facilities, have gained importance in recent years due to its potential as a renewable energy source. Poplar (*Populus* spp.) is one of the most commonly used species as an energy crop in southern Europe due to its high production in high density plantations and short rotation. Weed control is one of the main obstacles in the production of poplar for biomass, especially during the first year of crop establishment, period of maximum sensitivity to weed competition. In drip-irrigated poplar there are two types of problems resulting from the different position of weeds in relation with the crop. Weeds growing in crop rows cause major problems since they are favored by water and nutrients supplied by the irrigation system, competing strongly with trees. Weeds located on the inter-row area have a completely different ecology, since they are not able to use water from the irrigation system, competing with the crop slightly. In order to provide farmers with strategies for more effective weed management and reduced use of inputs, the objective of this study was to evaluate alternative weed management systems, in both inter-row and crop rows.

Two different experiments designed in randomized blocks with four replications were carried out in 2012 and 2013 in the CSIC experimental farm "La Poveda" (Madrid, Spain). Poplar was grown at high density: 3 m between rows and 0.5 m between plants. The first experiment included three management strategies in the inter-row: i) standard (preemergence oxyfluorfen + postemergence tillage): ii) herbicide-based (preemergence oxyfluorfen + postemergence glyphosate); and iii) based on cover crop (*Lolium multiflorum*). The second experiment involved eight management strategies in the crop rows: 1) glyphosate with protective hood; 2) glyphosate through a contact applicator; 3) string trimmer; 4) burning; 5) paper cover; 6) preemergence oxyfluorfen; 7) hand weeding; and 8) without control. Weed density and biomass and poplar production were assessed in all cases. Univariate ANOVA was used to test whether there were significant differences in both weeds and poplar yields according to management strategy (as fixed effect factor) and block (four blocks) as random effect factor. Differences among means were explored by Bonferroni post hoc test.

Results from the first growing cycle showed significant effects of weed management system in the inter-row, with more vigorous poplars in the herbicide-based strategy, while cover crop strategy decreased the production of poplar. These results were inversely related to the presence of weeds, with higher density and weed biomass in cover crop relative to the herbicide-based strategy. Furthermore, the study evidenced the necessity (and difficulty) to control weeds in the crop rows, as revealed in the treatment "without control" where poplar biomass was nil. The highest production was obtained with oxyfluorfen applied preemergence. However, this treatment resulted in poplar biomass 28 to 44% lower than in the hand weeding treatment. Glyphosate treatment using a contact applicator produced acceptable control of weeds, but resulted in reductions in poplar biomass production which ranged between 73% and 84% compared to the hand weeding control. The other strategies resulted in significant reductions relative to the control. Obviously, the results of both experiments should be considered as "not-definitive" since they belong to the first year in a crop cycle lasting three years.

# ECONOMIC EVALUATION OF MECHANICAL AND CHEMICAL WEED CONTROL ALTERNATIVES IN TRANSPLANTED CABBAGE

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Weed control options for transplanted cabbage (*Brassica oleracea* var. *capitata*) production were studied in Brodsko-posavska County, at village Slobodnica during two growing seasons. The objective of our research was to evaluate treatments efficacy and economic benefits of different weed management practices. The recommended post-plant herbicide application was compared with mechanical option, straw mulch and untreated control in a randomized complete-block design with four replications.

Post-plant application of herbicide metazachlor (Butisan S) was applied 10 days after transplantation, as recommended, with 2 l/ha. Mechanical inter-row cultivation was tested as a sole treatment three weeks after the cabbage were transplanted, and for mulch was used wheat straw applied immediately after transplanting the crop. Control plots without herbicides and mechanical soil disturbance were also established.

Throughout the season, the weed community was dominated by a few species that had high relative abundance value, while most of the species were of low abundance. Competing weeds prevent cabbages plants from accessing all the moisture and nutrients in the soil and caused weak or stunted growth. No significant differences were observed in weed control on plots with mulch and with herbicide application, as expected. They failed to control weeds and did not generally provide significant in-season weed suppression.

However, economic benefit from successful weed control option is what growers need. Yield from our experiments, together with crop production and market statistics, were used to determine the economic costs, benefits, and incremental rates of return from investing in weed control. In our analysis, mechanical option was a dominating treatment. With mechanical control weeds were significantly reduced, adequate yield were observed and what is more important mechanical control produced a highest net return.

## WEEDS AND WEED MANAGEMENT IN PEPPERS

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The EWRS Working Group "Weed Management Systems in Vegetables" was established with the aim of collecting and disseminating information and results on weeds and weed control strategies in vegetables, identifying gaps in knowledge and defining new research projects. So far the working group has published reviews on onions, tomatoes, carrots, peas, cabbages and lettuces. Information about key weeds, new weeds or species that have recently become problematic, effect of competition, weed management programmes in integrated and organic production, approved herbicides and those currently undergoing registration for use in sweet and hot peppers grown in Italy (I), Poland (PL), Portugal (P), Spain (E) was collected.

In 2012, the world production of peppers was 31 millions of tons on 1.9 millions of hectares. In the surveyed countries peppers crop surface is about 32750 ha (E 19000 ha, I 11000 ha, PL 2500 ha, P 250 ha,). The majority of the crop is transplanted. In Spain, direct seeding was studied and the results transferred to farmers but this technique has not been adopted. The season of planting is late spring (April and May). Single-row distance is 0.80 to 1.00 m, while double-row distance is 1.20 to 1.50 m, with a planting density of 3-4 plants m<sup>-2</sup>, depending on cultivars. Peppers suffer severe weed competition due to low initial growth rates. The critical period for weed control in transplanted crops was about from 25 to 45 days after planting. Polyethylene mulching and drip irrigation is generally used to avoid weeds and to optimize irrigation. Localised irrigation instead of sprinkler irrigation and fertigation instead of broadcast fertilisation can also help to reduce weed emergence and competition.

The weed communities are commonly very rich of species and their composition is highly variable in relation to climate, soil, crop rotation and crop period. The most important and frequent species are: *Cyperus rotundus, Datura stramonium, Xanthium strumarium, Portulaca oleracea, Solanum nigrum, Sonchus oleraceus, Picris echioides, Chenopodium album, Amaranthus spp., Portulaca oleracea, Polygonum spp., Convolvulus arvensis, Setaria verticillata, S. glauca, Digitaria sanguinalis, Echinochloa crus-galli, E. colona, Sorghum halepense. Some species are becoming important: <i>Diplotaxis erucoides* and *Malva sylvestris* in E, *Ambrosia artemisifolia* and *Amaranthus hybridus* in I, *Galinsoga* spp. in PL.

Conventional weed control is based on herbicide application; options are restricted to: glyphosate (E, I, PL, P), pendimethalin (E, I, P), oxadiazon (I), napropamide (E), clomazone (E, I) and graminicides (E, I). However, mechanical weed control (hoeing) is often used to compensate for poor herbicide efficacy. IWM generally involves: 1) false seedbed technique followed by shallow harrowing or by glyphosate application; 2) pre-transplanting herbicide application; 3) post-transplanting inter-row hoeing or rotary cultivation. Common strategy for organic production is: 1) false seedbed technique followed by shallow harrowing; 2) transplanting; 3) repeated inter- and intra-row cultivation (i.e. hoeing, split-hoeing and/or finger weeding) through the growing season; 4) hand-weeding. The use of starch-based biodegradable films are increasing, while other biodegradable mulching materials is under study. In Spain, paper is a promising candidate because it controls all weeds including *C. rotundus*, which pierces the plastic films.

# MANAGEMENT OPTIONS FOR MULTIPLE HERBICIDE RESISTANT PAPAVER RHOEAS IN SPAIN

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Papaver rhoeas L. is one of the most troublesome dicotyledonous weed in winter cereals, and since some decades ago the appearance of herbicide resistant biotypes to ALS inhibitors and/or synthetic auxines took place in several countries across Europe (Spain, Greece, France, Italy, Germany, Denmark, Sweden and United Kingdom). The development of integrated weed management (IWM) systems for this species is mandatory, especially under the new European Directive 2009/128/CE. In this sense different integrated management strategies were assessed in North Eastern Spain.

A trial has been conducted in the locality of Cubells (Lleida province) in a winter cereal field during two consecutive seasons (2013 to 2015), where previous dose-response experiments confirmed the presence of a multiple resistant *P. rhoeas* population to tribenuron-methyl and 2,4-D (resistance factors estimated 2311,3 and 14,5 respectively). Four management systems have been considered, all under two different tillage systems (no tillage and minimum tillage) in a split-plot design with three replicates. The management systems included crop rotation with sunflower, delayed sowing, high cereal densities or herbicide applications at different timings. Data was analyzed with Generalized Linear Models using R.

Initial weed densities were, on average, higher under direct drilling compared to minimum tillage (100 vs 20 plants per square meter). The management systems significantly most efficient in controlling *P. rhoeas* were those including a crop rotation with sunflower and/or early herbicide applications (0 plants per square meter at the end of the second season). Delayed seeding effectiveness was much dependent on weather conditions each season.

This trial is still underway for another third season, and the possibility to implement IWM systems to control multiple herbicide resistant *P. rhoeas* populations in winter cereals integrating chemical and non-chemical tools will be demonstrated. These integrated strategies are necessary, both to alleviate the resistance problem and to extend the life span of available herbicides.

# DIFFERENT FARMING MANAGEMENTS IN APPLE ORCHARDS: EFFECT ON PLANT SPECIES COMPOSITION AND DIVERSITY

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In the 21<sup>th</sup> century, an active conversion from conventional to the integrated and organic production systems in fruit orchards has been going on in the Czech Republic with aim to reduce environmental pollution, ensure better product quality and increase biodiversity.

The survey was performed in three triads of selected apple orchards in Moravia and in Central and Eastern Bohemia in the Czech Republic in 2009. In each Bohemian region, one conventional, one integrated and one organic orchard were selected. In Moravia, two organic and one integrated orchard were chosen. In each orchard, 30 permanent plots of 1 × 1 m were set up. Fifteen plots were located in tree-rows and fifteen between rows of apple trees. Three times in the growing season, species composition and cover of vascular plants were studied in plots. The mean number of species per plot (alpha diversity) and the total number of species occurring in all 30 plots in one orchard (gamma diversity) were subsequently calculated. Plants identified were classified on the base of their residence time into three groups; natives, archaeophytes, and neophytes. The effect of type of management and region were evaluated using CCA and Monte Carlo permutation tests. Both factors had a significant effect on changes in species composition of studied vegetation. The effect of regions with different soil and climatic conditions was found more important than the effect of management type.

The highest values of both alpha and gamma diversity were found out in organic orchards, followed by integrated and conventional ones. For all three compared regions, differences in diversity measures were found as statistically significant. Totally 201 vascular species were found; among them 127 natives, 65 archaeophytes, and 9 neophytes. As the most common archaeophytes, *Capsella bursa-pastoris*, *Senecio vulgaris*, and *Cirsium arvense* were observed; the most common neophytes were *Amaranthus retroflexus*, *Veronica persica*, *Oxalis fontana*, and *Conyza canadensis*. Share of native species varied between 49 and 87 % of the total number of species per plot, while archaeophytes and neophytes represented 7-36 % and 0-14 % per plot, respectively.

# TEAM-UP CROP DIVERSIFICATION AND WEED MANAGEMENT: PRODIVA

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The research-network PRODIVA focuses on a better utilization of crop diversification for weed management in North European arable cropping systems. The goal is to maintain diverse arable weed vegetation that is manageable in the long-term and could fulfil other necessary system-functions including support of beneficial organisms. The partners in PRODIVA will synthesize knowledge from terminated and running research projects and set-up selected new experiments on cover crops and variety resp. crop mixtures. Moreover, we will interact with partners from farming practice and extension services in organic agriculture. Regional fields will be surveyed for weeds to safeguard the relevance of the experimental research. Current cropping practices and their influence on weed pressure and weed diversity will be identified. The project will involve relevant stakeholders from the participating countries to assist in targeting the undertaken research. Project structure, aims and research methods will be presented.

Though PRODIVA has a primary focus on arable cropping systems in Organic Farming we are convinced that within the EU-legislative framework of IPM all arable farming systems can profit from the results. Neither are crop diversification methods restricted to Organic Farming, nor can IWM (Integrated Weed Management) be successfully implemented without respecting the role of weeds in agro-ecosystems.

The project "PRODIVA - Crop diversification and weeds" is supported within the ERA-net CORE Organic Plus transnational programmes (Project ID 1381).

# WEED SPECIES RICHNESS IN GENETICALLY MODIFIED GLYPHOSATE-TOLERANT MAIZE

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Genetically modified herbicide-tolerant (HT) crop varieties are not commercially grown in the EU. Our trial was approved by the Ministry of Environment of the Czech Republic. Seeds of GM maize were provided by Monsanto Company. Field trial with HT maize variety NK603 tolerant to glyphosate was established in 2013 in central part of the Czech Republic (Odrepsy, 50°9'17.183"N, 15°12'28.657"E, 205 m a.s.l.) in three blocks with different herbicide treatments, each consisting of following variants in three replicates: conventional ploughing, conservation tillage, mulch tillage with *Sinapis alba*, mulch tillage with *Trifolium incarnatum*, mulch tillage with *Hordeum vulgare* (winter barley), mulch tillage with *Phacelia tanacetifolia*. Only data related to different herbicide treatments are presented. Plot size was 540 m<sup>2</sup> (30×18 m). Herbicide treatments were as follows: 1: Conventional POST standard iodosulfuron-methyl-Na at 1.5 g/ha; foramsulfuron at 45 g/ha; isoxadifen-ethyl at 45 g/ha; 2: Split dose of glyphosate at 1080 g/ha crop BBCH 13 + 1080 g/ha crop BBCH 16-18; 3: POST acetochlor at 1170 g/ha; terbuthylazine at 556 g/ha + glyphosate at 1080 g/ha - crop BBCH 13-14. PZS 200 ST Sprayer, 10 m (Prodag, CZ) was used with 110-SF-04 nozzles, at a water volume of 200 l/ha. During crop vegetation, weed occurrence was recorded once per month. Data were analyzed by ANOVA.

The grain yield was not significantly different among all tested treatments, soil tillage systems and cover crops. The average yield of all the plots was 8.3 t.ha<sup>-1</sup> in 2013, lower compared to previous years and similar experiments. The main reason for this can be found in unfavorable weather conditions for maize during the first half of vegetation.

The weed occurrence expressed as percentage plant coverage was relatively high on plots of treatment 1 during the whole vegetation period. Lowest values of weed coverage were found in plots of treatment 3 in all evaluation terms. In total, 63 species of vascular plant were found. Major weed species were *Amaranthus retroflexus, A. powellii, Convolvulus arvensis, Echinochloa crus-galli, Fallopia convolvulus, Chenopodium album* and *C. hybridum*. Before post-emergent herbicide treatments, the weed species richness was similar under all the treatments. After the treatments, average numbers of weed species per experimental variant: were related to the total weediness; the highest numbers of weed species were recorded in plots of treatment 1.

# DOES REDUCED HERBICIDE USE AFFECT BIODIVERSITY AND CROP PRODUCTION?

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Weeds are both harmful for crop production and important for biodiversity. Herbicides pollute the environment, and thus French and European regulations require a drastic decrease in herbicide use. Because of the long-term survival of weed seeds in soil and because of the multiplicity of biological, environmental and agricultural conditions, models are essential to evaluate weed management strategies. Here we use an existing weed dynamics model to evaluate the effect of the intensity of herbicide use on agricultural production and weed-related biodiversity.

The FLORSYS model quantifies the effect of crop succession, management techniques and climate on multi-specific weed dynamics over the years (Colbach *et al.*, 2014) as well as weed impact on biodiversity and crop production (Mézière *et al.*, 2014). Farm surveys were carried out in two French regions to record agricultural practices, with farms ranging from organic to those with intensive pesticide use. The surveyed cropping systems were simulated with FLORSYS over 30 years and repeated 20 times with randomly chosen weather scenarios recorded in both French regions.

The simulations only showed a slight effect of herbicide treatment frequency index (TFI) on weed benefits and harmfulness for production and biodiversity. Weed species richness averaged over the rotation tended to decrease and weed-based trophic offer for domestic bees to increase with increasing TFI whereas weed species equitability as well as trophic offer fir birds and carabids were not affected. Weed-related yield loss and field infestation did not depend on TFI while harvest contamination and harvesting problems tended to decrease with increasing TFI. Conversely, additional crop disease increased with increasing TFI which tended to favour grass weeds susceptible to the pathogen. Organic farms did not perform better or worse than herbicide-based systems, and there was generally a large variability for a given level of herbicide use, resulting from variations in other cropping practices (e.g. rotation, tillage, sowing dates). The cropping systems combining the highest biodiversity performance with the lowest weed harmfulness and herbicide use were those with catch crops grown before at least 50% of the crops.

The present work was financed by INRA, the ADVHERB project (ANR-08-STRA-02), and the research programme "Assessing and reducing environmental risks from plant protection products" funded by the French Ministries in charge of Ecology and Agriculture.

Colbach N. et al., 2014. *Weed Research*, in press. Mézière D. et al. *Ecological Indicators*, 48, 157-170.

# ANALYSIS OF RELATIONSHIPS BETWEEN FARMING PRACTICES, WEED FLORA AND CROP PRODUCTION

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Quantitative data on the potential losses of crop yield due to weeds is limited. Approaches to quantify weed damages considered only individual weed species, and have not included the compounding of effects at the community level. Moreover they did not integrate the influence of agricultural practices and environmental variables on the weed-crop relationship. Here we analysed a big data sets to study relations between crop management, weed communities' indicators and crop yield and then develop path analysis models to disentangle the confounding effects of cropping system on weeds and crop production. Flora surveys (N= 651) were performed from 2006 to 2013 on 150 fields in the Fénay study site (Dijon, eastern France). Farmers were interviewed to know their cropping systems (N=564) and recorded their yield (N=564). The main crops were winter wheat (195 fields, 227 flora surveys), winter barley (94, 110), oilseed rape (92, 103) spring barley (53, 63) and mustard (45, 56). Data were also collected on the 11 others crops but on less fields (<25 fields, <27 flora surveys for each crop). Pearson correlations were performed between quantitative variables (farming practices, weed flora indicators) and yields.

On winter wheat, yield decreased with increasing total weed abundance (p-value = 0.01, r = -0.15) and weed species richness (p-value <0.001, r = -0.28) but was stable with decreasing weed community equitability (p-value = 0.58). Yield increased with the total number of farming operations (p-value <0.001, r = 0.30). These results suggested that more complex relations between crop management, weed species and crop yield are underlined here. We currently build a theoretical model and develop methods of partial least squares path modeling. These analyses will link sets of farming practices and flora indicators to crop yield and will quantify the strength of the direct and indirect relationships.

Keywords: yield, productivity, weed management, cropping systems, agronomy, agroecology.

Acknowledgements: This work was financially supported by the ANR project Agrobiose (ANR-13-AGRO-0001-03), the Burgundy department (FABER program, CouvHerbi project S. Cordeau). Flora surveys were performed during successive projects (ANR Systerra Advherb). Authors thank farmers for their helps during interviews.

# WEED SPECIES IN ROW AND INTERROW OF BORDEAUX VINEYARD FIELDS ACCORDING TO THEIR MANAGEMENT

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In recent years, grass cover of the inter-row in Bordeaux vineyard has largely increased (representing 80% of vineyard fields today). Sown or regenerated from the soil seed bank, it aims to compete with the vineyard for water and nitrogen resources to limit excessive vine vigour and improve wine quality. Moreover grass cover allows to reduce herbicide reliance and could protect soils against erosion by increasing organic matter content and biological activity.

However the grass cover can also severely compete with the vineyard and reduce vine yields in particular conditions: on deteriorated soils (lack of organic matter, compaction phenomenon), with a low available water capacity and in a context of climate change (more contrasted climate). Consequently, innovative soil management systems coupling grass cover establishment and soil fertility improvement strategies are necessary. This study aims at inventorying weed species in Bordeaux vineyard fields in rows and inter-rows with different management, in order to identify potential competitive weed communities with plant descriptors extracting from trait data-bases.

Flora surveys were performed in autumn and spring 2013 and 2014 on 14 fields located in Gironde and Dordogne departments (Western France). Walking surveys on at least four rows per field allowed to list all species observed in the rows and inter-rows. Frequency of occurrence and density were both evaluated for each species with specific scale (from 1 to 5, 1 = species found one time or isolated individual; 5 = species often recorded or in a dense patches). Each species was described by traits extracted from databases: botanical division, life span (annual/biennial/perennial), Grime strategies (stress tolerant/ruderal/competitive), Ellenberg's indicator values.

Flora surveys identified 207 different species and were mainly composed by perennials species (51%), with competitive ruderals (CR-species: 35.5%), stress-tolerant ruderals (SR-species: 25.7%) Grime strategies'. Most observed species are: *Plantago lanceolata, Trifolium repens, Lolium perenne, Potentilla reptans, Agrostis stolonifera.* Weed communities were dependent of soil management with on more representative species (frequency and density>4) with almost twice perennials than annuals species in the no-tilled interrow and as much annuals than perennials in the row.

Future flora surveys associated with characterizations of surrounding landscape, soil of fields (physico-chemical and organic parameters), cultural practices and agronomical vine performances (Ntester, petiole sampling, maturity indicators), will allow to highlight key factors influencing presence and distribution of floristic species in Bordeaux vineyard. The long term objective is to determine practices enhancing floristic communities allowing suitable vineyard field productivity.

Keywords: Vineyard, floristic inventories, perennial/ biennial/ annual species, Grime strategies.

#### INFLUENCE OF DIFFERENT TYPES OF TILLAGE AND HERBICIDES ON WEED INFESTATION AND CROP IN CORN MONOCULTURE CONDITIONS, FOREST-STEP ZONE OF UKRAINE

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Weed control in corn is one of the key elements of modern corn production systems including no-till systems. The acreage of corn under no-till systems is permanently increasing in Ukraine. At the same time, the potential risk of the appearance of resistant weed biotypes is a serious danger for weed control systems based on herbicides use. The goals of these trials were to gain insights into the long term efficacy of herbicides under different methods of soil tillage, possible changes in the weed flora and corn yield under the conditions of Forest-Step zone of Ukraine.

Field trials were carried out in 2009-2013. Three methods of soil tillage were investigated: 1. Plowing at a depth of 20-22 cm; 2. Disking at a depth of 10-12 cm; 3. No-till. Five concepts were used for weed control:

concept 1: application of acetochlor (1200 g a.i. ha<sup>-1</sup>) before the emergence of corn followed by glyphosate (1080 g a.i. ha<sup>-1</sup>) around the three leaf stage of corn.

concept 2: application of a tank mix of the herbicides mezotrione (150 g a.i. ha<sup>-1</sup>) and glyphosate (1080 g a.i. ha<sup>-1</sup>) (row spacing)<sup>-1</sup> after crop emergence.

concept 3: application of glyphosate (1080 g a.i. ha<sup>-1</sup>) after crop emergence followed by glyphosate (1080 g a.i. ha<sup>-1</sup>) at a later stage.

• concept 4: application of glyphosate (720 g a.i. ha<sup>-1</sup>(row spacing) after crop emergence followed by a tank mix of dicamba (240 g a. i. ha<sup>-1</sup>) and glyphosate (1440 g a.i. ha<sup>-1</sup>) at the 8 leaf stage of maize.

concept 5: usually applied conventional herbicide program topramezon (62.5 g a.i.ha<sup>-1</sup>) + dicamba (160 g a.i. ha<sup>-1</sup>) + metolat 1.25 l ha<sup>-1</sup>

No-till variants were treated every year before corn planting with glyphosate (1080 g a.i.ha<sup>-1</sup>). Conventional corn hybrid DKS-3511 was planted in all trials. Row spacing were treated with glyphosate, weeds in the row were hand-weeded.

The following weeds were predominantly recorded at the beginning of the trials: Annual grasses: Setaria glauca L. and Echinochloa crus-galli (L.) Roem.Broadleave weeds: Chenopodium album L., Amaranthus retroflexus L., Polygonum aviculare L., Polygonum lapathifolium L., Galinsoga parviflora L., Thlapsi arvense L. The total plant number was 184-196 plants/m<sup>2</sup> and Elytrigia repens L. -5-7 pieces/m<sup>2</sup>. Over the investigated 5 years the best control efficacy was observed in conception 1. Elytrigia repens disappeared in all glyphosate variants whereas Erigeron canadensis L. and Portulaca oleraceae L. started\_to appear. The composition of the annual weed flora did not change even under the no-till system in concept 2 where glyphosate was used 14 times during the test period.

Conclusion: the efficacy of the tested weed control concepts has not been sufficiently different against methods soil tillage. No tendency towards an decrease in efficacy was observed. Corn yield in no-till variants was lower than under plowing.

# WEED MANAGEMENT IN NO-TILL SYSTEMS GREATLY BENEFITS FROM LOW-DISTURBANCE SEEDING

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The germination of seeds is a key process in the dynamics of weed populations. In no-tillage fields, the mulch layer on the soil surface can lower the weed seed germination. However, the soil disturbance caused by seeders affects the seed dormancy status of most weed species, and induces the non-dormant seeds to germinate. Reducing soil disturbance during seeding has therefore the potential of diminishing the number of germinating weed seeds. Based on this premise, the coulter disks of a no-tillage seeder were equipped with metallic, ski-shaped blades, mounted on both sides of each disk. The blades were installed to reduce the soil disturbance and to keep the mulch layer in place, thereby minimizing soil exposition while performing the seeding in the field.

To evaluate the equipment, five experiments were conducted in no-tillage fields on a clay soil in the south of Brazil. Three experiments were carried out with soybean and two with maize. During the evaluation, soil exposition shortly after seeding, determined by means of digital image analysis, weed density and crop yield were determined. Additionally, in the maize experiments, herbicide efficiency was assessed.

The modified seeder reduced soil exposition in four of the five experiments, reducing the fraction of exposed soil up to 78% compared to that of the standard seeder. Only in a soil with a minimal mulch layer (<1.1 t ha<sup>-1</sup> of straw), the modified seeder did not reduce soil exposition compared to the standard seeder. In soybeans, the weed plant density in the crop rows was reduced with on average 55%, and in two out of the three experiments, weed plant density in between crop rows was also reduced. Accordingly, the use of the modified planter reduced weed biomass and increased soybean grain yield. In the maize experiments the equipment also reduced weed density, though this reduction was species specific e.g. *Bidens* sp. (55%), *Ipomoea* sp. (50%), *Brachiaria* [syn.: *Urochloa*] *plantaginea* (37%) and *Raphanus raphanistrum* (26%). Due to a lesser weed density when seeded with the modified planter, the efficacy of herbicides was increased. Based on these results it is concluded that the modified seeder is effective in reducing weed establishment in no-tillage systems, and it can be a valuable tool in a more environmentally-friend Integrated Weed Management strategy.

## SOIL CONSERVATION PRACTICES COMBINATION WITH LOW HERBICIDE USE TO CONTROL WEEDS IN GRAIN CROP SYSTEMS IN SWITZERLAND

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Soil conservation techniques are generalizing in field crop systems in Switzerland to limit soil degradation. They are accompanied by a weed issue that is largely handled with herbicides. To limit environmental concerns associated with such products, it is necessary to limit their use and to find alternatives. The good combination of the conservation techniques should be able to manage weeds. However, no tillage may be preferred to reduced tillage as far as soil protection is concerned in priority despite the better weed control potential of the latter. Besides, some cover crops, interesting against weeds, have uncertain development through years, depending on the soil, climatic or agronomic conditions. Other species compete weeds over a too short period due to their sensitivity to low temperatures. Finally, references are mostly limited to the study of one factor. The main objective of our study is to define weed control strategies involving cover crops, soil tillage and herbicides while reaching the best compromise between weed control, performance of the succeeding maize crop and limited use of synthetic herbicides. The first set of analyses, presented here, aims at finding cover crop species capable of controlling weeds in the less intensive weeding strategies.

To that purpose experimentations were implemented between 2010 and 2014, on different experimental fields each year, at the research station of Agroscope Changins (Nyon, Switzerland). In a first step, ten non-wintering cover crop species were sown in three randomized complete blocks after the harvest of a winter wheat. After the winter, the maize was sown in the residuals of frozen cover crops that had either i) been mixed within the first centimeters of soil with a rotary harrow or ii) left undisturbed on soil surface. These sowings were applied as sub-treatments to cover crops. Herbicides applications were integrated to soil tillage sub treatments. A risk assessment of the weed infestation was used to support decisions concerning these applications: i) before maize seeding in form of a total herbicide application, ii) in maize as post-emergence treatment. For each decision of herbicide application a control was set up, except in cases leading to definitively unsuccessful maize cultivation. This decision strategy allowed to compare different cover crops was monitored before winter. The soil cover of their residues and weeds were observed at the end of winter. Weed soil cover was observed during maize establishment. Performance of maize as silage was monitored at harvest.

During the four years of experimentation, totally skipping the use of herbicide was only possible in 2012. For the three other years, the herbicide was used according to the risk of infestation by weeds. In some situations it appeared a posteriori that the total herbicide was unnecessary as far as weed control is concerned. It was especially true when this treatment was associated with soil tillage. The analysis of cover crops in the different tillage and weeding strategies showed that almost all tested species were statistically as efficient against weeds in less intensive strategies as in more intensive ones. On the contrary, the intensive weeding strategies. The results of this study should help farmers to combine the best conservation practices to handle simultaneously several ecosystem services.

# **SESSION V**

# **NEW CHALLENGES**

# CLIMATE CHANGE AND WEED FLORA FROM A FENNO-SCANDINAVIAN PERSPECTIVE

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Elevated carbon dioxide concentration [CO<sub>2</sub>], and resulting climate change might influence agricultural weeds in Fenno-Scandinavia in several direct and indirect ways. Firstly, direct effects with factors as e.g., extended vegetation periods, milder winters and changed precipitation patterns might enable species at their range margins to migrate further north. Secondly, changes in cropping sequence, soil cultivation etc. and increased acreage of crops like maize, soya bean and sunflower would have indirect effects on weed flora and weed density.

At a global scale it has been argued that crops (mostly  $C_3$ ) will benefit more than weeds (mostly  $C_4$ ), since the advantage of  $C_4$  over  $C_3$  plants is expected to decrease with increased [ $CO_2$ ]. However, this would not be applicable on Fenno-Scandinavian conditions since the vast majority of both crops and weeds growing in that area are  $C_3$  plants. In addition, predicted summer droughts might benefit  $C_4$  weeds in two ways; i)  $C_4$  plants in general are more drought tolerant than  $C_3$  plants, due to less opened stomata, and ii) farmers might be more inclined to cultivate maize, a  $C_4$  crop with high drought tolerance. Cultivation of maize might open the temporal window for weed species with slow development, and in need of crops with a long growing period, e.g. the  $C_4$  species *Echinochloa crus-galli*.

Increased temperatures, mainly expressed as extended vegetation periods and milder winters, will enable a number of causal aliens to reproduce and establish viable populations. The impact on agriculture might however, at least in the initial phase, be marginal since many of these species are short day plants, i.e. they flower late in season when nights become longer. Thus, even if they are able to expand their range of distribution they will rarely be able to set seeds before crop harvest under Fenno-Scandinavian photoperiodic conditions. Nevertheless, it is not known to what extent genetic variation in traits regarding photoperiod requirements would enable a future adaptation to northern latitudes.

In summary, changes in weed flora and weed frequency could be divided into three categories:

i) agricultural weeds, which presently are geographically restricted within the region, might expand further north due to changes in climate and land use. Good examples are *Alopecurus myosuroides*, blackgrass, and *Bromus sterilis*, barren brome, two troublesome grass weeds in winter cereals.

ii) species with a relatively long presence in the flora, but with a short history or marginal occurrence as established agricultural weeds. They are typically linked to specific crops, with spatial or temporal windows open for these marginal weeds. Examples are *Echinochloa crus-galli* and *Amaranthus retroflexus*.

iii) well-known agricultural weeds in central and southern Europe, but rarely seen in the Fenno-Scandinavian flora (causal aliens). This includes the quantitative short day plant *Ambrosia artemisiifolia*. It has so far, due to its short day requirement for flowering, not been able to establish populations in the region.

# WEED CONTROL BY ALLELOPATHIC POTENTIAL OF CORN (ZEA MAYS L.)

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Suppression of certain weeds by allelopathic potential of crop plants is considered as a new technique in Iraq, attracting attention as alternative way for weed control. Allelopathic crops were used to reduce consumption of synthetic herbicides in heavy doses which created environmental pollution and developed herbicide resistance in some weeds. Allelopathy is an environmental friendly technique for controlling weeds and help in reducing cost of herbicides. Certain crops restrain growth of some weed species while releasing phytotoxic substances from their residues, inhibiting seed germination and growth of weeds. The allelopathic potential of corn (*Zea mays* L.) may play a crucial role in sustainable agriculture; the aim of the study is to investigate the potentiality of corn to be used as bioherbicide.

Laboratory experiment was conducted for seed germination, aqueous extract concentration (0, 3, 6%) was used, and green house experiments for plant growth, residues concentrations (0, 3, 6 gm / kg soil), residues decomposed for different periods in the soil (0,15, 50,100, 150 gm/ kg soil), and root exudates of (*Zea mays*) were used against three weed species: (*Amaranthus retroflexus* L., *Echinochloa colonum* (L.) Link. *and Cyperus rotundus* L.).

The results indicated that the aqueous extracts of corn significantly reduced seed germination and plant growth of all studied weeds, the highest reduction for plant growth recorded was 54.6% and 48.6% for *A. retroflexus* and *E. colonum* respectively when 6% of aqueous extracts was used.

Residues of corn also inhibited the growth of weeds significantly; the inhibition in *A. retroflexus* was 62.4% and 51.4% when 3, 6 g residues / kg soil was used. Root exudates of *Z. mays* induced 27.6% and 29.8% significant reduction in the growth of both *A. retroflexus* and *E. colonum* weeds, respectively, while the root exudates of corn significantly promoted the growth of *C. rotundus* which increased more than 83%. The effect of residues decomposed in the soil on growth of *A. retroflexus* in the start weeks of the experiment was more than the end weeks of the experiment. The highest inhibition (78.9%) of plant height was observed after 6 weeks of decomposition when 150 g of residues / kg soil was used, generally high reduction of seed germination% of *A. retroflexus* was noticed between week 2-6 of decomposition periods, and by increasing the concentration of residues in the soil, the inhibition rate was increased.

The results of analysis of phytotoxins by HPLC indicated that four types of inhibitors; vanilic acid, syringic acid, p-coumaric acid, and ferulic acid were present in the residues of *Z. mays* decomposed for different periods in the soil. From these results it could be concluded that the corn plant has potentiality to be used as bioherbicide for suppression of tested weeds.

Keywords: Bioherbicide, corn, weeds, Allelopathy, residues, decomposition, aqueous extracts.

## LET'S TAKE ADVANTAGES OF *EUCALYPTUS GLOBULUS* PLANTATIONS FOR WEED CONTROL IN SUSTAINABLE AGRICULTURE!

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*Eucalyptus globulus* Labill. (Tasmanian blue gum) is widely grown in European forestry exploitations. In Spain, eucalyptus plantations cover around 325,000 ha and they are mainly devoted to produce cellulose pulp. The abundant eucalyptus harvest residues, by-product of the paper industry, as well as the evidences of the allelopathic nature of this forest species, led us to propose the use of eucalyptus leaves as green manure for weed control in cropping systems [1].

In the present study, two-year field experiments were conducted to probe the potential of *E. globulus* leaves for weed control in weed-infested maize fields from two different locations. Fresh eucalyptus leaves were slashed in small pieces and incorporated into the soil at the dose of 2,2 kg m<sup>-2</sup>. Control treatments consisted of eucalyptus-free plots. After a safety period of fifteen days after eucalyptus incorporation to overcome the initial phytotoxicity caused by allelopathic compounds released from the plant material [1], maize was sown. Samples were harvested at 1, 3 and 6 months after eucalyptus incorporation.

Significant reductions in weed biomass were observed in the two locations and years. Eucalyptus leaves could significantly control broadleaved weed species such as *Solanum nigrum* L., *Chenopodium album* L., *Artemisia vulgaris* LINN., *Raphanus raphanistrum* L. and *Galinsoga parviflora* Cav. Weed biomass of monocotyledon species was also reduced by eucalyptus green manure, being affected species such as *Digitaria sanguinalis* (L.) Scop., *Holcus mollis* L. and *Cyperus rotundus* L. Phytotoxic effects were more pronounced at early stages of maize establishment, reducing growth competition between crop and weeds. Our results constitute evidence that the incorporation of *E. globulus* harvest resisues to soil can be a feasible practice to reduce the reliance on synthetic herbicides in maize-based cropping systems.

[1] Puig CG, Álvarez-Iglesias L, Reigosa MJ, Pedrol N (2013). Weed Science 61: 154-161.

#### ABUTILON THEOPHRASTI – FROM A WEED TO A CROP FOR MODERN USE OF RENEWABLE RESOURCES

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Abutilon theophrasti is one of the most important weed species in various regions and summer annual crops in the world. However, in the past *A. theophrasti* (ABUTH) was grown – and is still grown in China - as a crop for fiber production. Other fiber crops and mainly fibers made from crude oil as resource substituted ABUTH fiber in most cases. Due to the positive effects of the use of natural fibers, like reduced weight, lower costs, lower carbon footprint and better opportunities of recycling at the end of product life, fiber containing plants should be investigated for technical use. Focus should be on fiber yield and quality as well as use of by-products. ABUTH is very unassuming towards environmental conditions and can be grown on poor soils. Furthermore, ABUTH has a strong and persistent fiber. Aims of our studies are: How to grow ABUTH as a crop (plants/m<sup>2</sup>; row distance, N-fertilization, weed control)? What is the (fiber) yield? How is the quality of the fiber compared to other fiber crops? In a second part of our project ABUTH fiber should be converted to a fiber-reinforced composite. These materials can be used for several purposes like car in-cabin room. Quality of ABUTH based products should be compared to products that are already on the market.

Field experiments showed that ABUTH can be grown in Germany and yield is about 10 t/ha (dry matter). Fiber content is about 15%. Grain yield as by-product was near 1 t/ha. ABUTH seeds contained 20% oil with high (65%) portion of C18:2. However, many seeds shed to the ground before harvest and increased soil weed seed bank. This may reduce the possibilities to integrate ABUTH in several crop rotations, where ABUTH is hard to control (like sugarbeet). Fiber quality of ABUTH was comparable to hemp (*Cannabis sativa*). However, ABUTH fiber is in some physical properties different from hemp fiber (like: elongation at rapture, less uptake of moisture). Furthermore ABUTH by-products after fiber extraction are more valuable than those from hemp. The by-products can be used for feeding (seeds) or technical uses (stems for chips trays or insulation material). Field trial results showed highest yield with 30 plants/m<sup>2</sup> and with a row spacing of 50 to 60 cm. Selective weed control in ABUTH is possible by applying sugarbeet herbicides based on Metamitron and Phenmedipham tank mixtures.

However, ABUTH is a serious weed and seed fall could not be avoided completely if the plant is grown as a crop. Consequently, one part of our studies is to investigate influence to crop rotations and soil tillage on ABUTH seed bank in the soil and occurrence of volunteers in following crops.

First results of growing ABUTH as a fiber crop look very promising and investigations are ongoing with the focus on use of fibers for technical purposes. If useful applications of ABUTH by-products can be found, it might be an interesting crop in some crop rotations especially on poor soils.

## USE OF ALLELOPATHIC PLANTS AS LIVING MULCH IN APPLE ORCHARDS

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Living mulches obtained by plants with allelopathic effects are among choices in sustainable weed management. However, effect of living mulches can change depending on soil and climatic conditions of the area. A field study with *Festuca rubra* L., *Trifolium repens* L., *Trifolium subterraneum* L., and *Agrostis tenuis* Sibth., whose allelopathic effects have been documented in the literature, was conducted in an apple, cv red Fuji, orchard in the age of 4 years in the South Marmara Region of Turkey. The main weeds in the site were *Sonchus oleraceus* L., *Cynodon dactylon* (L) Pers., *Convolvulus arvensis* L., *Cyperus rotundus* L., *Anagallis arvensis* L., *Galium aparina* L., *Sinapis arvensis* L., *Sorghum halepense* (L) Pers., and *Papaver rhoeas* L. Soil was prepared and seeds of living mulches were sown in October 2009. In addition to living mulch treatments, two check treatments were established: One is weedy check and the second one is the common farmer application which includes tillage twice in March and May followed by glyphosate application in 5-6 leave stages of weeds.

The number of weeds was reduced by all applications compared to weedy check. The highest weed control (94 %) was obtained by the current farmer weed management practices. The ratio of weed control by living mulches changed between 73 % (in case of *T. subterraneum*) to 60 % (*A. tenuis*). The apple yield was not statistically different in 2011, but in 2012 the use of *T. subterraneum* gave the best weed control and apple yield among the living mulches used, comparable with current farmer application. *T. repens* also gave better yield than weedy control but yield in the application of two other species was the similar with weedy check. *A. tenuis* seems have adaption problem for the region. *T. subterraneum* and *T. repens* can be recommended as living mulches for weed control in the South Marmara Region of Turkey. These options can help organic apple producers as well as traditional ones. Living mulch application can be considered a method to prevent/delay herbicide resistance.
#### "VRAIES MESSICOLES": A LABEL FOR RARE ARABLE PLANTS PRODUCED FROM THE WILD FOR FUNCTIONAL AND ORNAMENTAL PURPOSES AS A WAY TO PRESERVE BIODIVERSITY

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Sowing seeds of rare arable weeds might be an opportunity for both preserving threatened plants adapted to arable habitats and contributing to farmland biodiversity providing valuable ecosystem services like pollination. Dramatic changes in the plant communities of arable lands have occurred during the past decades, mainly due to land use intensification such as chemical weed control. In Europe, many weed species and even whole weed communities are on the brink of extinction. In order to stop this decline, the French Department of Ecology set up a national program to preserve rare arable weeds with two main purposes, (1) to preserve still existing wild populations and (2) to restore biodiversity by sowing seeds of local provenance.

In France, floral mixtures for arable wildflower strips and landscape architecture currently include seeds of unknown or non-local origin. They might be horticultural varieties or distant populations of rare arable plants. Sown in the fields, these commercial seed mixtures threaten local diversity. Introduced ecotypes may be more competitive than local ones and their spread may result in cryptic invasions. Hybridization and genetic introgression may reduce the fitness of local populations. To improve this situation, the National Federation of Botanic Conservatories (Fédération des Conservatories botanique nationaux), and its partners implemented a national system for certification of wild seed collection and propagation:

- identification of seed transfer zones: areas within which plant material can be exchanged without disrupting genetic patterns or local adaptation;

- strategies to track the origin of plant material, from wild collection (unknown origins are not accepted), to propagation in stock and final use;

- guidelines to preserve biodiversity during collection and propagation

- set up of a national system for certification of native plant species, including a step by step control procedure of seeds and plants produced.

The quality trademark "Vraies messicoles" (genuine rare arable weeds) provided by the certification system will thus encourage the production and availability of native species, and should protect wild populations from genetic introgressions. The trademark is integrated in a more general programme providing guidelines for the use of native plant material under the label "Végétal local" http://www.fcbn.fr/vegetal-local-vraies-messicoles. In contrast to the umbrella label, "vraies messicoles" allows the production of rare species and emphasizes a close collaboration of producers and users in an initiative to preserve the threatened biodiversity.

## CONTROL OF AILANTHUS ALTISSIMA IN A NATURAL ENVIRONMENT

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Ailanthus altissima (Mill.) Swingle (commonly named tree of haven, family: Simaroubaceae) is a very dangerous invasive plant species. The invasiveness of this dioecious species is due to its ability to reproduce, equally well, both by seed (one plant can produce thousands of "flying" samaras) and asexually (its extended and vigorous root system generates numerous suckers and progeny plants). The species is able to adapt to any type of soil and water regime. It spreads everywhere in urban and sub-urban areas, on roadsides, railways and ruins, in uncultivated or abandoned areas, gardens and green spaces. A. altissima plants reach the heart of natural areas causing severe ecological effects. Plants form highly dense stands outcompeting native species and reducing their growth. Thus this species represents a serious threat both in natural and anthropic areas.

The management of *A. altissima* is very difficult. The most common methods include manual, mechanical and chemical control. Hand pulling can be carried out only on very young seedlings before the root system has developed. Mechanical removal (cut) is the most used technique in public areas but proved to be costly and quite ineffective, as it induces a faster development of suckers and resprouting shoots. Moreover, in urban and archeological areas the use of mechanical equipment can be very dangerous or even not practicable. Spray treatments of herbicides are frequently not allowed in urban and natural areas due to health and environmental risks.

The Alta Murgia National Park is a very wide Park (over 68,000 ha) located in the Apulia Region (Southern Italy). It is a Site of Community Importance (SCI) and a Special Protection Area (SPA) within the EU Natura 2000 network. Within the Park, *A. altissima* is the most spread invasive plant species, and it is considered one of the most serious threat for the biodiversity.

A project named "LIFE Alta Murgia" was funded in 2013 by the European Commission within the LIFE+ Framework, aimed at eradicating *A. altissima* from the Alta Murgia National Park by using innovative and eco-friendly control techniques, based on a minimized use of effective herbicides with environmentally friendly stem applications (e.g. stem injection, cut stump, spaced cut). In order to accurately take a census, map and quantify the infestation in the whole park, plan the interventions, manage the control program and check the progresses of the management practices, a *ad hoc* software was initially developed.

In the present communication the procedures used and the results obtained in the weed mapping will be shown. Moreover, the first results regarding the control treatments and their effectiveness will be presented.

#### INVESTIGATING A POTENTIAL AUXIN-RELATED MODE OF HORMETIC/INHIBITORY ACTION OF THE PHYTOTOXIN PARTHENIN

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The phytotoxin parthenin is a plant metabolite that is biosynthesized by the invasive weed *Parthenium hysterophorus* L. and is believed to contribute to the invasiveness of the weed *via* allelopathy. Investigating the dose-response characteristics of parthenin confirmed an allelopathic potential, however, at low doses parthenin proved to significantly stimulate plant growth. The observed hormetic action was speculated to be auxin-like and, therefore, the present study investigated the hypothesis of an auxin-related hormetic action of parthenin and whether the inhibitory action may differ from low-dose effects. The experimental strategy comprised two approaches: (1) joint action dose-response experiments with *Lactuca sativa* L. and (2) dose-response experiments with auxin/antiauxin-resistant genotypes of *Arabidopsis thaliana* L. Heynh. The observed patterns of occurrence of hormesis along with potential shifts of dose-response curves provided an indication for an auxinic mode of action.

The joint action approach was based on the concept that biologically inactive doses of the auxin IAA will impact the expression of hormesis and shift a dose-response curve if both, the stimulatory and the inhibitory response is due to an auxin-related action of the mixture partner. Therefore, dose-response experiments were conducted as germination assays with *L. sativa* treated with parthenin alone or mixed with sub-inhibitory doses of IAA. Trying to establish this approach with another auxin (IBA) and known auxin-antagonists (PCIB, TIBA) showed, however, that biologically inactive IAA doses did not consistently shift the response and partly lowered the upper asymptote of the dose-response curve. As hormesis is prone to fail under conditions of retarded growth, this approach seems challenging to investigate hormesis in general. Nevertheless, as parthenin in mixture with IAA did not significantly differ from the biphasic solo treatment in two independent experiments, this approach provided no indication for an auxin-related action of parthenin.

The second approach used two auxin-resistant (*aux*1-7, *axr*2) and two antiauxin-resistant (*aar*1-1, *aar*3-1) mutants of *A. thaliana* in comparison to the sensitive wildtype (Col-0) in complete doseresponse experiments with parthenin and two reference compounds with known auxin-related action (IAA, PCIB). IAA induced hormesis only in *aar*1-1 and showed significantly right-shifted curves for the auxin-resistant mutants and *aar*1-1. PCIB showed significantly right-shifted doseresponse curves and hormesis only in antiauxin-resistant mutants. Hence, the inhibitory doseresponse patterns of the two reference compounds widely confirmed previous reports, while the observed promotion of PCIB-resistant mutants indicated that the hormetic mode of action of PCIB may differ from its antiauxinic inhibitory action. Parthenin induced hormesis in sensitive and auxin/antiauxin-resistant genotypes and showed an inhibitory response pattern considerably differing from that of IAA and PCIB. Therefore, this approach also provided no indication that the hormetic or the inhibitory mode of action of parthenin may be auxin- or antiauxin-like.

The study showed that due to the complex nature of hormetic effects and its usually variable occurrence, traditional experimental approaches tracing the mode of action may face some limits. Therefore, the results obtained in this study are insufficient for a final conclusion, however, the hypothesis of an auxin-related stimulatory or inhibitory action of parthenin could not be confirmed.

#### ALLELOPATHIC POTENTIAL OF GOLDENROD (SOLIDAGO VIRGAUREA L.) TOWARDS GERMINATION OF SOME CROPS

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The Genus Solidago belongs to Asteraceae familly. There are about 100 species derivered mostly from North America. In Poland the Genus is represented by 5 species including only 2 native taxons: *Solidago virgaurea* L. and *Solidago alpina* L. All Solidago Genus plants are characterised by anemochorical diaspora dispersal and its seeds easily germinate in the wide range of soil types. These plants are also able to vegetative propagation and that ability promotes their intensive expansion in different areas and zones. The most widespread species of the Genus are: *Solidago gigantea* Ait. and *Solidago canadensis* L. and however they are present in Poland for many years they obtained status of invasive species. An alternative explanation of invasive species rapid distribution is the release of different allelocompounds. There are many scientific papers regarding *Solidago canadiensis* allelopatic properties but there is very little evidence of allelpatic features of *Solidago virgaurea* in scientific literature.

The aim of experiments was to evaluate the allelopatic potential of Solidago virgaurea on germination of some crops i.e. *Hordeum vulgare* L., *Fagopyrum esculentum* Moench, *Zea mays* L., *Sorghum vulgare* Perz. and *Secale cereale* L. All the tested crops are also known for their allelopatical properties.

In order to determine the allelopathic potential of aquatic liquids obtained from *Solidago virgaurea* the phytotoxicity test based on seed germination and early growth was used. The test is based on measures of young roots during several days of exposition and it can be done by computer software. Only young leaves of *Solidago virgaurea* were used in the experiment. Four water extract concentrations of *Solidago virgaurea* were assessed: 0 g of leaves/10 ml distilled water (dw.), 0,5 g of leaves/10 ml dw., 1 g leaves/10 ml dw. and 2 g leaves/10 ml dw. Transparent containers with 90 ml of quartz sand (granulation 0,2 - 0,8 mm) and paper filters were covered by seeds of tested crops. There were separate containers for each species, and substrate of each container was moistened with distilled water (30 ml). Containers with seeds incubated vertically in  $25^{\circ}$ C.

Seed germination energy/capacity and number/mass of roots of all the tested crops were assessed in the experiment. Our research suggests that *Solidago virgaurea* had strong allelopatic potential towards germination and initial root growth of tested crops. Data indicated that concentrations 1:10 and 2:10 caused the greatest inhibition effect on germination and roots fresh weight. Each of *Solidago virgaurea* concentrations reduced roots fresh weight of *Zea mays* and *Sorghum vulgare*. *Hordeum vulgare* and *Secale cereale* responded to the applied concentration by the reduction of germination energy and capacity, and roots fresh weight reduction as well.

## PHYTOTOXIC COMPOUNDS FROM VOLATILE EXTRACTS OF CYTISUS SCOPARIUS AND C. STRIATUS

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The behaviour of *Cytisus scoparius* (L.) Link as highly successful invader in many countries worldwide led us to explore its allelopathyc nature. *C. scoparius* and its close relative *C. striatus* (Hill) Rothm are abundant in the Atlantic shrubland, where they are native species. In the search of new natural products and materials for weed control in sustainable agroecosystems, in this study we determined the chemical volatile profile of these two species and bioassayed the phytotoxicity of its compounds in order to assess their potential as bioherbicides.

The chemical composition of the volatile extracts of flowering aerial biomass of *C. scoparius* and *C. striatus* (collected in SW Galicia-Spain) were analyzed by GC and GC-MS. Twenty compounds were identified from the *C. scoparius* extract and twenty-seven from the extract of *C. striatus*, both representing more than 95% of the total extract.

Twelve volatile compounds present in these extracts were tested separately for their phytotoxicity by bioassaying their *in vitro* effects on the seed germination and early growth of *Amaranthus retroflexus* L. and *Digitaria sanguinalis* (L.) Scop. Volatile bioassays [1] were performed by testing the compounds at 0, 6.25, 12.5, 18.17 and 25 ppm in the sealed Petri dish atmosphere.

The results showed that most of the monoterpenic compounds strongly inhibited both germination and growth of the tested target species, being  $\alpha$ -terpineol and eugenol the most phytotoxic compounds for *D. sanguinalis*. Verbenone revealed as an effective bioherbicide, especially against *A. retroflexus*.

Keywords: broom, weed control, GC-MS, bio-herbicide, monoterpenes, phytotoxicity.

[1] Vokou et al. (2003). J. Chem. Ecol. 29: 2281-2301.

#### DIGITARIA SANGUINALIS-USTILAGO SYNTHERISMAE PATHOSYSTEM: IS THERE ANY VARIABILITY IN THE SMUT INFECTIVITY AND THE PLANT RESISTANCE?

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Throughout the world there are great extensions of crops in which reducing chemical and/or mechanical weed control might be desirable; alternative or complementary methods for weed management, such as biological control, are needed. *Digitaria sanguinalis* (L.) Scop. is a widely distributed summer annual weed, even in glyphosate-tolerant summer crops, and it is sensitive to *Ustilago syntherismae* (Schwein.), a smut that can transform its inflorescences into masses of spores. In annual plants affected by sterilizing smuts avoidance of infection is crucial, while in the event of infection, the existence of different degrees of tolerance could affect the interaction dynamics. In turn, infection is crucial for smuts that cause monocyclic diseases entering through seedlings; variation in infectivity and aggressiveness could also affect the disease dynamics. In order to plan any biological control strategies, information is needed about the variability as in plant qualitative and quantitative resistance, as in fungal infectivity and aggressiveness, because the disease dynamics could be largely dependent of this items.

An experiment was performed with *D. sanguinalis* spikelets vacuum inoculated with ustilospores of *U. syntherismae*, both collected in 2011. The objective was to explore the importance of two factors in the proportion of infected seedlings: the type of spikelet (formed on non-smutted healthy plants SH, and formed on partially smutted plants SP) and type of ustilospore (formed on plants with all the inflorescences smutted US, and formed on partially smutted plants UP) with the aim to elucidate if the fungal infection event depends much more on the seed source of variation or on the spore source of variation. Inoculated spikelets were observed for germination. The seedlings were covered for 48h with silver foil and incubated for at least 10 days at 20°C 12h darkness/30°C 12h light, cleared and stained. If internal hyphae were observed seedlings were considered to be infected. Analysis of the infection frequency was performed using generalized linear models of binomial distribution (complementary logit link function and Type III analysis options) considering both factors (type of spikelet and type of ustilospore) and the interaction between them. The least square means of the levels of the effects were computed and compared using probability values from the chi-square distribution.

Results showed that the type of ustilospore was the only significant effect (P<0.0001, n=934). Spikelets inoculated with UP spores were less infected (49%) than those inoculated with US spores (89%), whether the spikelets were SH or SP. Percentage of infection of all combinations were: US x SH and US x SP (79% and 88% respectively P=0.1160 when compared), UP x SH and UP x SP (50% and 48% respectively P=0.7702 when compared). Thus, spores formed on partially smutted plants were, on average, much less infective than those formed on completely smutted plants. Variability in the spikelets' resistance was not detected; spikelets formed on apparently healthy plants were as resistant as spikelets formed on partially smutted plants to the two types of ustilospores. Our results showed clearly that the introduction of the *U. syntherismae* populations in a field with the aim to reduce the *D. sanguinalis* population densities will be much more efficient if the sources of fungal inoculum are spores from completely smutted plants rather than spores from partially smutted plants.

## FUNGAL PATHOGENS OF THE GENUS *DIAPORTHE* AS SOURCES OF NOVEL HERBICIDAL COMPOUNDS

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Saffron thistle (*Carthamus lanatus* L. ssp. *lanatus*, Family: Asteraceae) native of Southern Europe, is one of the worst and widespread winter-growing annual weeds of pastures and crops throughout Australia. Poor results of mechanical and chemical control have made this weed a suitable target for biological control. Efforts to develop a mycoherbicide against this weed led to isolate pathogenic strains of *Phomopsis* sp. from naturally diseased saffron thistle plants in Australia, and to evaluate their potential as mycoherbicides against the host. More recently the teleomorph of this pathogen was identified as *Diaporthe gulyae* on the basis of morphology, DNA sequence analysis and pathology studies, and it proved to be pathogenic also to sunflower (*Helianthus annuus* L.), causing severe stem cankers.

Bioactive fungal metabolites have been long considered for their potential direct use as natural herbicides, as a lead for new herbicides or to discover novel mechanisms of action.

A study was then undertaken to ascertain the ability of *D. gulyae* to produce phytotoxic metabolites *in vitro*, and to evaluate their potential as natural herbicides. Two other *Diaporthe* species, namely *D. kongii* and *D. kochmanii*, both pathogenic to sunflower, were also considered in this study.

The fungal strains were grown on defined mineral liquid medium and the eventual toxicity of the culture filtrates was assayed on host and non host plants. The bio-assay guided purification of the filtrates allowed to obtain several novel and interesting compounds, whose chemical structure was determined, or is in progress by using spectroscopic, chemical and chiroptical methods combined with X-ray analysis.

In particular, a novel phytotoxic geranylhydroquinone, named phomentrioloxin, was firstly isolated from the culture filtrates of *D. gulyae* as the main phytotoxic metabolite. It was characterized as (1*R*,2*R*,3*R*,4*R*)-3-methoxy-6-(7-methyl-3-methylene-oct-en-1-ynyl). More recently, a SAR study was performed on phomentrioloxin by preparing seven derivatives of the toxin, assayed for the phytotoxic, antimicrobial, and zootoxic activities. A further investigation of the culture organic extracts of the same fungus led to the isolation of further four new metabolites, named gulypyrones A and B, and phomentrioloxins B and C, whose chemical and biological characterization is in an advanced stage. A new 3-substituted-5-diazenylpentendione, named kongiidiazadione, was identified from the culture filtrates of *D. kongii* together with the already known toxic 3-nitropropionic acid. Finally, a number of known and novel compounds has been purified by the culture filtrates of *D. kochmanii*, whose characterization is in progress.

The present communication will report the latest results of the chemical and biological characterization of the known and novel compounds isolated from the fungal cultures of the three *Diaporthe* species mentioned above.

## MODELLING THE DAMAGE NICHE TO ESTIMATE FUTURE WEED PROBLEMS UNDER CLIMATE CHANGE

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Distribution ranges of plant species are changing in reaction to rising temperatures and changing precipitation patterns. There is a general shift polewards, with some species expanding their range and others losing area. Ecologists have been using environmental niche models to estimate range shifts and future species distributions. This method uses records of species occurrence together with recent environmental data to describe the environmental niche, i.e. the environmental conditions under which a species can establish and reproduce. In combination with data from climate models future suitable habitat distribution can be projected.

The occurrence of a species does not necessarily mean it will cause damage in an agricultural crop. For weed management, we therefore extended the modelling approach to estimate the damage niche, i.e. the environmental conditions under which a weed species can establish and causes damage, for example yield loss.

To demonstrate the approach, we modelled potential species distribution of nine major European maize weed species in Northern Germany: *Calystegia sepium* (L.) R. Br., *Chenopodium album* L., *Echinochloa crus-galli* (L.) P.Beauv., *Fallopia convolvulus* L., *Polygonum aviculare* L., *Setaria viridis* (L.) P. Beauv., *Solanum nigrum* L. and *Viola arvensis* Murray. Maize cropping is increasing strongly in the region due to rising climatic suitability and use as a source of renewable energy. Typical maize weeds are thermophile, spring germinating and drought tolerant. It is hypothesized that these weed species will profit strongly from rising temperatures, decreased summer precipitation and longer growing seasons.

MaxEnt was used to train A) models of the environmental niche using species records and environmental data from all over Europe and B) models of the damage niche with the same data set but reduced to the European regions where the species were classified by experts to cause major damage (Meissle et al., 2010). These models were then used with data of a regional climate model for the period 2070-2100 to project future distribution of environmental niche and damage niche.

Results indicated that the study region has suitable conditions for most of the species, at least in parts, but only three of the nine maize weeds are likely to cause major damage in the region: *Ch. album, E. crus-galli* and *P. convolvulus*. Only small changes in the suitable area were projected for the future, but the number of species which potentially cause major yield losses increases to six (including *C. sepium, P. avilvulare* and *S. viridis*), indicating that thermophile maize weeds will profit from climate change.

Meissle et al. (2010). J. Appl. Entomol. 134(5), 357 - 375.

# HOW TO EVALUATE THE EFFECT OF FIELD NEIGHBOURHOOD ON WEED FLORA AND ITS IMPACT ON AGRICULTURAL PRODUCTION AND BIODIVERSITY WITH A MODEL

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Weeds are a harmful pest for agricultural production but essential for biodiversity in agricultural landscapes. They must be managed with a long-term perspective, because their seeds survive for several years in the soil and their dynamics depend on numerous factors (cropping system, pedoclimate). Moreover, weed seeds disperse among fields, and the dynamics in one field can be influenced by agricultural practices in neighbouring fields as well as by adjacent semi-natural habitats. Consequently, weed management strategies are often evaluated and designed with models.

Here, we adapted the weed dynamics model FLORSYS (Colbach *et al.*, 2014) to include (1) several adjacent fields, (2) sown grass strips and road verges, (3) weed seed dispersal by natural vectors (Thomson *et al.*, 2011). We ran simulations on clusters consisting of 4-9 fields and grass strips. We compared maize-based cropping systems, differing in rotational crop diversity, mouldboard ploughing and tillage frequency, herbicides etc. The first results allowed us to link weed species progress in the landscape to species dispersal traits, indicating that weed densities in the neighbouring fields were more important for rapid migration than the dispersal mode (e.g. anemochory or zoochory), light seeds or tall weed species.

Current and prospective maize cropping systems identified from experts and data bases (Bürger *et al.*) were simulated. The weed contribution to biodiversity (species richness and equitability, trophic offer for birds, carabids and bees) varied less among years for a field cluster than for individual fields. Thus, low production and biodiversity in one field were compensated by higher performances in neighbouring plots. Simplified rotations reduced weed-related biodiversity but also harmfulness for production (yield loss, field infestation, harvest contamination). Conversely, no-till favoured biodiversity but increased harmfulness.

The present work was financed by INRA, the research programme "Assessing and reducing environmental risks from plant protection products" funded by the French Ministries of Ecology and Agriculture, and the EU project AMIGA (FP7-KBBE-2011-5-CP-CSA).

Bürger J. et al. *Agricultural Systems*, in press. Colbach N. et al., 2014. *Weed Research*, in press. Thomson F.J. et al., 2011. *Journal of Ecology*, 99 (6), 1299-1307.

#### INFLUENCE OF SHADING ON THE PHENOLOGICAL DEVELOPMENT OF THE INVASIVE WEED AMSINCKIA MICRANTHA

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Amsinckia micrantha is an invasive species which is spreading rapidly in Scandinavia and is becoming an increasing problem. In order to investigate why it has succeeded as a competitive weed we have investigated its phenotypic and physiological plastic responses to shading in green house experiments.

Plants were subjected to 0, 30, 50, 80, 90 and 95% shading. *A. micrantha* exhibited both phenotypic and physiological plasticity by achieving a higher chlorophyll content index per unit leaf area, stomatal conductance, photochemical efficiency, plant height, total dry matter and early flowering to partial shade stress (30%). The growth of *A. micranta* was substantially reduced by dense shading (80, 90 and 95%). Shading resulted in early flowering, and directed a large fraction of photosynthates towards storage for seeds production. We observed minimum low light seedling mortality in *A. micrantha*.

Our study showed that *A. micranta* may acclimatize to new sunny fields (photo-inhibition) and partially shaded places (under-stories). It may recover from reduced photosynthetic photon flux density (partial shade) because of morphological and physiological plasticity by increasing plant height, leaf area and regulated stomatal conductance as intrinsic traits under partial shade stress. It seems to use this plasticity as an adaptive mechanism to handle partial shade stress.

Keywords: Invasive, phenology, photosynthetic response, plasticity, shade stress.

#### IMPACT OF AN INVASIVE VINE (HUMULUS JAPONICUS) ON RIPARIAN VEGETATION

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Originating from Eastern Asia, *Humulus japonicus* Siebold & Zucc. (Cannabaceae) has been introduced in France for ornamental purposes at the end of the 19<sup>th</sup> century. While only few casual populations were recorded during the whole 20<sup>th</sup> century, *H. japonicus* is now established with invasive populations spreading along the river banks of the river Gardon (SE France) since at least 2004. While it may be obvious that invasive populations have an effect on the native vegetation, the magnitude of the impact, the identity of impacted species as well as the resilience of impacted communities are all key aspects to consider whether management actions are relevant or not. The aim of this study was therefore to measure the impact of *H. japonicus* on the diversity and composition of riparian communities.

These impacts were measured using 60 1m<sup>2</sup> quadrats equally distributed in three types of areas: i) areas invaded by *H. japonicus* (I), ii) areas where *H. japonicus* was formerly invasive but where its seedlings were removed at the beginning of the growing season (R) and iii) non-invaded areas in the upper parts of the river where *H. japonicus* could potentially established but where it has never been recorded (NI). The cover of each species was recorded four times during the growing season, in mid-March, mid-April, mid-May and at the end of July 2014. Differences in species composition were analyzed through Principal Coordinates Analyses (PCO) based on Bray-Curtis distances followed by a PERMANOVA to assess if species differences were significant among treatments. Species richness (S) and Pielou's evenness index (J) were computed and used as response variables in generalized linear mixed models where the areas (I, R, NI) and the dates were considered fixed factors and plot identity nested within sites were considered random factors.

At the beginning of the growing season, species composition was similar in I and R plots and significantly different from NI plots, but in the course of the season, R plots tended toward NI plots in species composition. There was a significant effect of treatment, date and their interaction. Species richness was significantly lower in I plots compared to R (*z*=-4.24, *P*<0.001) and NI (*z*=-2.73, *P*=0.017) plots while it was similar between R and NI plots (*z*=1.47, *P*=0.306). Species richness in invaded areas was reduced by more than 60% compared to non-invaded plots, which is a high impact for an annual species. Pielou's evenness index decreased as well in I plots (J=0.3) compared to both NI (J=0.6) and R plots (J=0.8).

Although plots where *H. japonicus* had been removed seemed to recover rapidly, the species composition is still rather different compared to 'non-invaded' plots due to other invasive species becoming dominant (*Artemisia annua, Helianthus tuberosus*) whereas native species, such as *Alliaria petiolata* or *Agrostis stolonifera* dominated the 'non-invaded' plots. This may indicate that *H. japonicus* invaded communites that were already disturbed.

Long-term investigations will be needed to monitor whether the communities impacted by *H. japonicus* are resilient at the longer term.

#### AMBROSIA SPECIES IN THE CZECH REPUBLIC AND THE FIRST FINDINGS OF FIELDS HEAVILY INFESTED WITH A. ARTEMISIIFOLIA

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In the Czech Republic, three species of the genus *Ambrosia* can be found. All of them are classified as neophytes. *A. psilostachya* DC. is a rare casual, only one site with its occurrence is currently known. It was first recorded in 1999, and as a perennial species, it still survives, with no greater tendency to spread. *A. trifida* L. was recorded for the first time in 1960; several locations for it are known, especially in urban and industrial areas, near railways *etc.* Many recorded populations are not stable, the only one under our monitoring had 12 individuals in 2013 and none in 2014. The most common species of this genus with the longest history in the Czech Republic is *A. artemisiifolia* L., which was found for the first time in 1883. At that time, it was imported as an impurity in clover seeds from North America. It began to spread during the second half of the 20<sup>th</sup> century. Its occurrence was restricted to railway stations, river ports and storage facilities for imported agricultural products. Only rarely was it also found on arable land, but only temporarily.

This situation changed in 2013 when we found two fields in the same region (Kolín district, Central Bohemia) with heavy *A. artemisiifolia* infestation. The weed occurred in maize stands. In the first case, there was a dense patch with *Ambrosia* in one corner of the field and individual plants scattered along the field border. In the second case, the whole field was heavily infested with Ambrosia plants, sometimes overgrowing maize plants (the height of Ambrosia plants was 1.0-1.5 m with some growing up to 1.8 m while the crop was weak because of unfavorable conditions at the beginning of its growth). In both cases, we contacted the farmers, gave them leaflets with basic information about *A. artemisiifolia*, publications focused on weed biology and management, and catalogues of herbicides available for weed management on arable land, together with instructions and recommendations for successful *A. artemisiifolia* management (including changes in crop rotation *etc.*). We applied non-selective systemic herbicide to minimize seed production at the beginning of seed ripening (time of application was related to the date of the first record of *Ambrosia* occurrence). Both farmers informed us that in 2014 they would establish cereal crops on the fields, with minimum probability of *Ambrosia* occurrence.

During monitoring in 2014, we found that the first farmer was taking good care of the field, with mulched field margins and no *Ambrosia* occurrence. The second farmer contrary to our expectations planted potatoes on a smaller part of the field and the crop was again infested with *A. artemisiifolia.* Fortunately, the weed plants were destroyed before full flowering during crop harvest.

## SPREADING OF INVASIVE SPECIES UNDER THE INFLUENCE OF REDUCED TILLAGE

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Aggressive penetration of invasive species influencing biodiversity is a threatening factor. These species can spread through natural formations of green-corridor just as ways developed by humans. The goals of the study were: (1) to reveal which invasive species exist in corn fields and (2) whether different ways of reduced tillage can affect the cover of invasive species.

The study was conducted in large-scale farming in Hungary, where traditional cultivation with ploughing was compared with three ways of soil cultivation without any ploughing, namely direct sowing, disk-tiller cultivation, and shallow cultivation in spring. The subjects of the comparison were the weed communities developed under each soil cultivation conditions. Random sampling was applied to gain weed cover data in fixed quadrants, which were processed by using ANOVA.

The rankings of invasive species coverage are the next: as for disk tiller  $(8,8\%) \rightarrow \text{direct}$  sowing  $(7,3\%) \rightarrow \text{shallow}$  cultivation  $(4,5\%) \rightarrow \text{ploughing} (0,04\%)$ . Disk tiller cultivation and direct sowing produced significantly higher weed cover compared to ploughing. Comparisons of yearly weed cover data resulted in similar rankings. Over a three year period, of the ways of reduced tillage shallow cultivation used in springtime produced significantly lower weed cover value than those of the other two. In the research field five invasive species appeared, namely: *Amaranthus retroflexus, Amaranthus powelii, Ambrosia artemisiifolia, Conyza canadensis, Xanthium italicum*. Each of them is invasive neophytes.

Amaranthus species constantly exist under all three reduced tillage, although, there is a decrease in their cover year by year. Disk tiller cultivation favours red-root pigweed (6-8%), while common ragweed preferred both plots cultivated by direct sowing and disk tiller cultivation. *Conyza canadensis* appeared in direct sowing only, producing a tiny cover which disappeared by the end of year three. *X. italicum* was detected under direct sowing conditions only.

Contrary to the most of the papers stating that invasive weeds spreading currently are largely of Mediterranean origin, we have found that their portions stagnate or diminish on the research fields. In short, we state that a higher cover of invasive species can be envisaged for cultivation without ploughing, although for maize production, for which use of herbicide is still an integral part of the production technology, the invasion of those weeds is to be slacked.

#### EARLY DETECTION AND ERADICATION OF INVASIVE SPECIES IN POZEGA VALLEY, CROATIA

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Invasive species have imposed enormous economic and ecological costs upon ecosystems and the services they provide to humans. During the beginning of invasion process they often consist of a small number of colonists, such that the cost of excluding colonists is trivial compared to dealing with the problem after populations become established and spread.

Management of nonindigenous, invasive plant species is among the most challenging undertakings of natural resource managers. Successful management of invasive species often requires an integrative approach, which combines monitoring, research, control tools, preventive regulations and interagency coordination. Information on the distribution and abundance of nonnative plant species is particularly important for large conservation lands where new introduction or expansions of existing weed populations may be difficult to detect in a timely and cost-effective manner.

A detailed survey was conducted in order to prevent new introductions and the secondary spread of existing nonnative species in Pozega valley, located in central Slavonia region in the Republic of Croatia. During the investigation, a total of 28 nonindigenous plant species were recorded. Their occurrence data were used to generate non-native plant invasiveness rank together with the data of their ecological impact, biological characteristics and dispersal ability, ecological amplitude and distribution and difficulty of control. In order to reduce the dimensionality of the above data set and thus to explain the relations between variables, the multivariate statistical method of factor analysis is used in order to transform the original variables to fewer variables. These new variables (called factors) can be viewed as latent variables explaining the joint behavior of nonnative species.

Risk assessment for nonindigenous plants in Pozega valley identifies 11 species: Ailanthus altissima (Mill.) Swingle, Ambrosia artemisiifolia L., Conyza canadensis (L.) Cronquist, Eleusine indica (L.) Gaertn., Erigeron annuus (L.) Pers., Galinsoga parviflora Cav., Phytolacca americana L., Robinia pseudoaccacia L., Solidago canadensis L., Solidago gigantea Aiton. and Artemisia annua L.

Taking no action for management these species could result in significant environmental and economic impact for investigated region.

#### EFFECT OF CHANGING CLIMATE ON GROWTH AND CONTROL OF THE INVASIVE WEED BROMUS TECTORUM L. BY GLYPHOSATE

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Changes in climate are supposed to improve the growth of invasive weed species. Further, knowledge gaps exist regarding the efficacy of herbicides under changing climates. We simulated four different climatic conditions in closed chambers. These included: 1. Control or ambient environment (CO<sub>2</sub>-concentration = 400-450 µmol m<sup>-2</sup> s<sup>-1</sup>; temperature 20/10 °C day/night): 2. High temperature (CO<sub>2</sub>-concentration = 400-450 µmol m<sup>-2</sup> s<sup>-1</sup>; temperature 25/15 °C day/night): 3. High CO<sub>2</sub>-concentration + high temperature (CO<sub>2</sub>-concentration = 800-900 µmol m<sup>-2</sup> s<sup>-1</sup>; temperature 25/15 °C day/night): 4. High CO<sub>2</sub>-concentration (CO<sub>2</sub>-concentration = 800-900 µmol m<sup>-2</sup> s<sup>-1</sup>; temperature 20/10 °C day/night). The growth of the invasive weed species *Bromus tectorum* L. was evaluated under these conditions. In order to find the effect of glyphosate under changing climatic conditions, glyphosate (0, 360, 720, 1080, 1440, 2880 g a.i. ha<sup>-1</sup>) was applied to control *B. tectorum* under either of the climatic conditions in the experiment.

Our results indicated that higher  $CO_2$ -concentration increased the growth of *B. tectorum* over control. Higher temperature was found to have a negative effect on the growth of *B. tectorum* while a combination of high temperature + high  $CO_2$ -concentration had increased growth of *B. tectorum*. Plant height, leaf length, fresh weight, dry weight, leaf area and gas exchange of invasive *B. tectorum* were positively affected by high  $CO_2$ -concentration and high  $CO_2$ -concentration + high temperature, while these variables were negatively affected by high temperature. Chlorophyll index and number of leaves were not affected by the different climatic conditions in the experiment. High temperature was found to improve the efficacy of glyphosate against *B. tectorum* while high  $CO_2$ -concentration + high temperature decreased the effectiveness of glyphosate against *B. tectorum*.

In conclusion, the increasing levels of  $CO_2$ -concentration and temperature had different effect on growth and control of this invasive weed species. High temperature decreased the growth and improved the control of *B. tectorum* by glyphosate application while high  $CO_2$ -concentration improved the growth and decreased the efficacy of glyphosate against *B. tectorum*. Positive effects of a combination of high  $CO_2$ -concentration + high temperature probably resulted only from the high  $CO_2$ -concentration.

Acknowledgements: Thanks to TÜBİTAK and BAP (ZRF-14015) for supporting the PhD studies of Khawar Jabran.

# THE DESTRUCTIVE INVASION BY AILANTHUS ALTISSIMA (MILL.) SWINGLE OF THE EIGHTEENTH CENTURY FORTRESS "CITTADELLA" OF ALESSANDRIA

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The *Cittadella* of Alessandria, north-west of Italy (44,9197° N; 8,6082° E), is one of the best preserved military fortifications present in Europe. Built in XVIII century it extendeds on an area of 60 hectares. In 2014 the citizen's movement "Europa nostra" has included the *Cittadella* in the "7 Most Endangered" (http://www.europanostra.org/2014-list/) program, which identifies the monuments and sites at risk in Europe. Since the abandonment by the Italian Army in 2007, the fortress underwent a gradual degradation. In this condition several herbaceous and wooded species colonized most of the buildings, increasing damages. *Ailanthus altissima* is the most troublesome among these species because of the high ability of the root system to infest building elements (roofs, walls) and roads, causing severe deterioration of the structures. The invasive potential of *A. altissima* is due to its abundant seed production by female individuals and high germination ability, its fast growth, the numerous suckers and the release of allelopathic compounds limiting the growth of other plants.

In 2013 a survey of the *A. altissima* presence in the *Cittadella* fortress was carried out to determine the level of infestation. The number of *A. altissima* plants present at *Cittadella* was assessed on three distinct areas: the bastions and curtain walls, the courtyards and internal roads, and the building roofs. The infestations were sorted in isolated plants, small groups of plants, linear infestations along the curtain walls, and large areas of infestation (>5m<sup>2</sup>). Overall, 32 isolated or small groups of plants, 26 linear infestations, and 8 large areas were identified. Linear infestations summed about 730m, while large areas were spread on about 7200m<sup>2</sup>, with an average infestation of 10-13 stems/m<sup>2</sup>. Male plants represented the majority of the infestations, and few female plants were present on the bastions.

In order to prevent the spread of *A. altissima* and preserve the buildings from other damages, the eradication is undelayable. Different control strategies have been suggested and consist of an integration of mechanical (cutting, girdling) and chemical means. Hand pulling of young seedlings and suckers should be avoided on the buildings to prevent further damages on the structures. Considering the high level of infestation detected and the high risk of resprouts, the chemical control should be preferred. Stem injection, cut stump and basal bark application of systemic herbicides are the most suitable options.

#### FLAME WEEDING: A NEW APPROACH IN WEED CONTROL FOR ARCHAEOLOGICAL SITES

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Archaeological sites around Greece are colonized by herbaceous communities. The presence of spontaneous, unmanaged vegetation, poses a threat to the monument. There is no official protocol of dealing with vegetation management in Archaeological sites, in Greece. However the national law prohibits the use of herbicides, as they can cause damage to the monument and to the environment and suggests the use of alternative methods of weed management.

The Agricultural University of Athens has undertaken a research program entitled: "Integrated management of vegetation at archaeological sites to protect monuments and enhance the historical landscape". One of the project's aims is to develop innovative methods for weed management in Archaeological sites that are friendly towards the environment. We applied flame weeding by different rates and number of applications, in order to examine the most efficient way to manage the weeds in such a fragile environment.

Three experimental fields were set up, in archaeological sites: Early Christian Amfipolis, Ancient Messene and Kolona in Aegina island. The experiment involved two rates of propane of 2 and 3.5 bars and two, three or four time application of flaming, every 14 days. The flamer that was used in the experiment was PiroBag-One by Maito, with a 30 cm wide flamer. Flaming treatments were applied for 52 sec per experimental plot (4 m<sup>2</sup>). Visual ratings of percent weed control were taken at 1,13,14, 27, 28, 41, 42 and 72 days after the first treatment. Height of twenty random plants per plot was also measured at 13, 27, 41 and 72 days after the treatments, while the dry biomass was measured from a 50 by 50 cm *quadrat* in sampling at day 72, after the treatments.

The overall control of vegetation increased as dose and number of applications increased. Similar results were obtained from all sites, where four time application of high rate, provided a nearly 100% control level through the growing season. Average plot height was significantly lower in flamed plots than non flamed ones. In Amfipolis, the average weed population height in untreated plots reached 140 cm at day 72, whereas four times treated plots, presented an average height below 20 cm. Dry weight reduction of biomass collected from the plots increased as number of applications increased. In the case of Kolona in Aegina dry weight reduction of four times application was above 90% and around 80% for three times application for both high and low rates of propane. The applied protocol of four time flaming of high rate, produced an acceptable level of spontaneous vegetation control and therefore it is proposed for use in archaeological sites, as an alternative method of weed management, where the fragile landscape is limiting our options.

## PESTICIDE RETENTION BY WEEDS DURING SUMMER FALLOW: DEVELOPMENT OF A NEW INDICATOR OF WEED IMPACT

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Weeds are usually considered as harmful for crops but they are essential for biodiversity. Weed management based on herbicides can pollute the environment and affect water quality. We thus need new cropping systems optimizing all cultural techniques, reconciling agricultural production, herbicide reduction and biodiversity conservation.

Model-based multifactorial approaches are considered necessary to evaluate and improve existing cropping system. Indicators were already developed to evaluate the cropping system in term of weeds impact on harvest, (such as yield losses), and biodiversity or nitrate losses by leaching. The effect of weed cover on nitrate trapping during summer fallow is well established but few studies are available on the temporary storage of herbicides by weeds during this period.

We thus propose here to develop a new indicator in order to integrate the contribution of weed cover on the decrease of herbicide leaching during summer fallow and to connect it to the weed dynamics FLORSYS (Colbach et al., 2014). The new indicator will be used i) to evaluate existing cropping system in terms of weed impact on the environment, ii) to design new cropping systems for sustainable management of the weed flora ensuring crop production while limiting impact on the environment.

The new indicator will translate the weed flora simulated by FLORSYS into an impact on the environment by weighting weed densities present during summer fallow by their ability to temporarily trap applied herbicides. Assuming a simple representation of pesticide root uptake based on transpiration stream concentration factor, the potential trapping of herbicides will depend on the lipophily of herbicides and weed transpiration. Indeed, these models are widely used in the literature to estimate the pesticide uptake by plant. Parameters related to pesticide lipophily and plant transpiration will be thus estimated from literature.

The new indicator will then be used to evaluate existing cropping systems identified in farm surveys and from expert opinion. These systems will be simulated with the FLORSYS-indicator association. Regression trees will then be used to identify combinations of cultural practices resulting in different levels of indicator values, e.g. crop production and herbicide trapping. The existing cropping systems will then be improved step-by-step in order to optimize selected indicators.

Acknowledgements: The present work was financed by the research programme "Assessing and reducing environmental risks from plant protection products" funded by the French Ministries in charge of Ecology and Agriculture.

Colbach N. et al. Weed Research, in press.

#### BEHAVIOURAL ECOLOGY OF WEED REGULATION BY CARABID BEETLES

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Farming relies heavily on the use of pesticides in order to maintain or even increase crop production. We now know, however, that over-use of pesticides has a strong and unwanted impact on the environment and on biodiversity. Thus, alternative solutions to better balance production and the environment have begun to emerge. The use of naturally-present predators of pests, such as ground beetles, for biological control is one of them.

Ground beetles exhibit a diversity of feeding regimes (diets) ranging from strictly feeding on seeds to being generalist predators (seeds and animal prey). Since they mostly feed on the seeds of weeds and invertebrate pests they appear a good candidate for biological control. How they choose, or switch, between their prey types and how this choice is influenced by the environment and/or the personality of the individual predator is still not clearly understood. This is the aim of my Ph. D. thesis.

I will start by looking at ground beetle food preferences using a two-stage experimental process. Initially, "no-choice" tests of food experiments will be done, which will be followed by "choice" tests in order to disentangle preference between different food types. We will look at how long it takes for one individual to find a prey and eat it with and without choice and if their choices are consistent when they have the choice. These tests will also allow us to investigate any possible competition that may exist between the two types of food, e.g. seeds and "meat", and thus the potential for an antagonistic effect between these two ecosystem services. We will then repeat these experiments in more complex environments, e.g. mesocosms, and introduce intraspecific competition to test the robustness of our preliminary food preferences results. The role of learning and personality will also be examined in relation to food choice and constancy.

#### SPATIALLY VARIABLE CORRELATIONS OF BARLEY YIELD WITH INFESTATIONS OF BLACK-GRASS (ALOPECURUS MYOSUROIDES) IN BARLEY FIELDS

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Weeds such as black-grass (*Alopecurus myosuroides* Huds.) often occurs in dense patches in arable fields. It is well known that crop yield is known to decrease with increase in weed density. These impacts on yield have been compounded by the development of herbicide resistance as well as the removal of approval for use of herbicides. Some farmers have therefore abandoned crops in parts of fields with particularly severe infestations by spraying with glyphosate to prevent production of viable seed.

At Reading we have been mapping spatial variability of black-grass within fields with a view to site-specific weed management. This paper reports on more detailed studies which were carried out in two uniformly-managed barley fields near Reading in the 2013 and 2014 harvest seasons in which crop yields, black-grass infestations and a range of soil characteristics have been investigated on a spatially variable basis in order to understand the spatial scales at which variation occurs and their correlations. An unbalanced nested sampling scheme was utilised with 138 small plots assessed in 2013 and 150 in 2014. Plots were separated by five spatial scales over the range of 1-55 metres in 2013 and 1-80 m in 2014 to assess variability and correlations at different spatial scales. Geostatistical analyses were used to derive variograms and kriged maps. Variance at each spatial scale was also analysed and correlations analysed. In this paper, the data is explored partly to understand the link between the incidence of the weed and soil type but also to investigate the strength of the correlation between yield loss and black-grass density at the different spatial scales investigated in each field.

Black-grass population densities varied greatly even over the finest scale of one metre, though more clearly over the widest scales of 55-80m. Correlation between black-grass population densities and soil moisture was unclear over short distances but positive (0.48) at the widest spatial scale of 55 m. Correlations at each spatial scale with crop yield are presented and discussed in the poster.

#### THE OCCURRENCE OF WEED SPECIES IN FIELD CROPS AND SEED MATERIAL

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Farming with an intensive system of production leads to fast extinction of more vulnerable and rare species of weeds, while organic or extensive farming fosters their occurrence. This study was conducted in cultivated cereal fields in south-east Poland for 6 years, from the beginning of the shift from conventional farming to organic farming. The aim of the study was to establish weed species composition in farmlands and in samples of cultivated plants material (collected before sowing and after harvest).

In the vegetation seasons of 2008–2014, fields of cereal cultivation at 12 farms were monitored. The variability process of weeds occurrence in the cultivated plant seed material was observed through field planting until field harvest. The number of weed species present in the seed material and in the material collected directly from the harvest was determined and a list of weeds species present in cultivated fields during vegetation season was compiled.

In the first years of monitoring, a small number of weed species was observed in the pre-sow and after-harvest material, with high frequency of more common species, such as: *Galium aparine*, *Polygonum convolvulus*, *Cirsium arvense*, *Setaria glauca*, *Chenopodium album*, which appeared in nearly each of the 12 samples with high abundance coefficient. In the following years, the number of weed species in the material obtained from the farmers grew gradually. In the last years, the differences in abundance between common and rare weeds gradually diminished. Abundance of more common species decreased slowly due to the appearance of other, less common, species. Seeds of these species came, most probably, from the soil seed bank. What is important, single specimens of rare species appeared in the cultivated fields, however, their seeds were not observed in the collected material yet. The results also show that not all weed species present in the field pass to the seed material, which means that their occurrence in the field increases the diversity, but does not reduce the quality and quantity of the harvest.

It can be expected that after years of cultivation without the use of herbicides, the vanishing weed species present in the soil seed bank will start to germinate, thus creating the full composition of various weed communities becoming extinct. Higher number of various species of segetal plants in cultivated fields means an increase in species diversity of fauna, which is indispensable for the balance in agroecosystems to be maintained.

#### TROUBLESOME WEEDS IN SPAIN: SURVEY TO FARM ADVISORS IN 2014

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During March to May, 2014, a questionnaire was designed using Google Forms with 44 multipart questions to collect data relating to farm advisor's demographics (6 questions), weed control (12 questions), information sources (6 questions) and most concerning weeds in specific crops (20 questions). The questionnaire was uploaded in June and invitations to respond were sent to advisors in all counties of Spain. By October 1st, 315 advisors had completed the questionnaire. Each advisor was asked to identify the most troublesome weeds for up to five crops, thus providing a total of 974 votes for the most troublesome weed candidates.

Local influence was evident, as 48% of answers came from Andalusia (Southern Spain). However, no differences were detected between Andalusia and the rest of Spain, at least concerning weed related issues. The analysis showed that farm advisors are as concerned about weeds as they are about other crop protection problems, i.e. pests or diseases. The weed threat was higher in annual crops (dryland and irrigated) compared to orchards and vines.

When asked about their current weed control means, only a minority was not satisfied with the efficacy (19%) or with the cost (14%). More worrisome was the reliance on agrochemical companies: 42% of advisors were confident that herbicide companies would provide solutions if new weed problems occur. Overall, chemical weed control is the most frequently used control method and is considered highly effective. When solutions are needed to manage a weed problem, it appears that technology transfer is very efficient for herbicide companies and quite inefficient from public institutions.

When analyzed, the 974 opinions on the most worrisome weed by crop provided the following as the top ten weed genera (in this order): *Conyza, Cyperus, Bromus, Lolium, Avena, Centaurea, Chenopodium, Echinochloa, Phalaris and Malva. Conyza* votes came mainly from advisors working with perennial crops. *Cyperus* was reported to be a problem in rice (probably *C. difformis*) and in irrigated crops (probably *C. rotundus*). Similar crops were affected by *Echinochloa*, but the species classification is more difficult. When asked what the criteria was for classing a weed as worrisome, the main criteria provided was surprising: difficulty to control was more relevant than crop yield reduction.

We found the survey very exciting and the results give insights into new challenges, mainly from the point of view of extension services.

#### WHICH DECISION-SUPPORT SYSTEM FOR SUSTAINABLE WEED MANAGEMENT: NEEDS AND CONSTRAINTS OF CROP ADVISORS

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Weeds are a major pest of crop production and play roles in supporting a larger biological diversity. We must change the traditional weed management, because we need to decrease herbicide use, because of pollution and weed resistance. Designing cropping systems reconciling these often antagonistic goals is difficult, because weed dynamics are influenced by numerous and interacting cultural practices whose impact depends on pedoclimate.

The mechanistic weed dynamics model FLORSYS (Colbach et al., 2014) is currently used to experiment current and prospective cropping systems on both aspects of weeds. The next step is to develop from FLORSYS simulations, via metamodeling, a simpler and faster tool for advisors to design innovative cropping systems for sustainable weed management and taking into account weed impacts on crop production and biodiversity. Because involving users in the design of decision support tools is crucial (Cerf et al., 2012), the objective of the present study was to identify the requirements and constraints of the future users of this tool.

A survey was conducted via an online questionnaire sent to advisors from regional agriculture councils and technical institutes from all over France. This inquiry was previously tested with some weed management and crop experts. The survey included four parts in order to identify: (1) the interviewee; (2) the aims, the contents and the structure of the decision-aid tool (e.g. which rotations? which dates?), on criteria for evaluating cropping systems (weed harmfulness, resource for pollinators...), on temporal aspects (one year, one rotation...) and on farming practices (list of operations in the field, decision rule...); (3) the constraints for model use, i.e. on the availability and difficulty to fill in the different types of input variables (crop succession, weeding tools...); (4) the functionality and readability of inputs and outputs of the future model, the ability to understand why a given input lead to the resulting output.

The questionnaire was put online (<u>https://enquetes.inra.fr/index.php?sid=31774&lang=fr</u>) and is still available. To date, 21 answers were received. A descriptive analysis will be performed on these answers. The results of this study will be essential to transform the complex mechanistic FLORSYS model into a simpler and easier-to-handle decision-aid model that answers to the requirements of its future users, which improves the probability that the new tool will indeed be used by advisors for designing sustainable cropping systems.

The present work was financed by INRA (EA and MIA divisions), the French project CoSAC (ANR-14-CE18-0007) and the Burgundy Region.

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## ESENIAS-TOOLS: A CONCRETE REGIONAL ACTIVITY

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ESENIAS (East and South European Network for Invasive Alien Species) was established in 2011. One of the main activities of ESENIAS has been the organisation of yearly workshops (Zagreb 2010, Sofia 2011, Belgrade 2012, Çanakkale 2013, and Antalya 2014) aimed at facilitating the participation of invasive alien species (IAS) experts from the participating countries, as well as other practitioners. The objective of the workshops was the setup of the network, the launch of the main activities, and the discussion of IAS issues of regional concern.

As a key result, a number of initiatives have started, including for the development of a database, needed to raise awareness and manage IAS in the region. The network is also active in facilitating the implementation of regional activities on IAS in accordance to the provisions set by the new EU Regulation on IAS No. 1143/2014. In this context, a new project has been just launched: "East and South European Network for Invasive Alien Species – a tool to support the management of alien species in Bulgaria (ESENIAS-TOOLS)". This initiative, funded under the Programme BG03 "Biodiversity and Ecosystem Services" within the EEA FM (2009-2014), will result in networking and development of IAS tools within the framework of ESENIAS to support the management of alien species in Bulgaria and in the overall region. New introductions of plants and other organism have been recorded in Europe and worldwide. These introductions, along with climate change and unsustainable farming practices will cause the appearance of new weeds in managed and unmanaged areas. We expect this one-year project to contribute also to the weed research and management in the East and South European countries.