



# **18<sup>th</sup> European Weed Research Society Symposium**

## **EWRS 2018**

17-21 June 2018  
Ljubljana, Slovenia

**New approaches for  
smarter weed management**

**Book of Abstracts**

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## **KEYNOTE LECTURES**

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## **GMOs and EU Economics and Policies**

Justus Wesseler

Wageningen University, Netherlands

The Opinion of the Advocate General (AG) of the Court of Justice of the European Union (CJEU) on the 'mutagenesis exception', triggered by the release of herbicide tolerant crops in France, in Directive 2001/18/EC illustrates how the current regime of genetically modified organism (GMO) regulation for release into the environment will deal with the regulation of New Plant Breeding Techniques (NPBT). The opinion is non-binding, providing only a proposal to the CJEU on how to solve cases. The Court historically has followed most of the Opinions of the AG, rendering it likely that also that of AG Bobek will be taken up by the CJEU and any issues raised by it will remain a concern.

One of the important clarifications the AG made is that it is not for the Court to interpret or adjust the Directive to address new scientific challenges to GMO regulation. This is rather the task of the legislators, either at EU or Member State level. This implies the EC has to come-up with a 'solution' how to address NPBTs.

The presentation discusses possible outcomes of the opinion and the implication this will have for the development of NPBTs including those used for developing herbicide resistant crops. The presentation considers past experiences of decisions made by the European Commission to draw conclusions about what to expect for the future.

## **Weed management in Middle Way agricultural systems**

Adam Davis

USDA-ARS, URBANA, United States of America

Public expectations of agriculture continue to grow, including not only abundant food production, but protection of environmental and human health, social justice and sustained profitability. Concurrently, production approaches have also become more polarized (e.g. organic/conventional, till/no-till, corporate/artisanal). Such orthodoxies are appealing in their simplicity, but can also create myopia that leads to unanticipated problems. Excessive reliance upon repeated soil disturbance for physical weed control in organic production systems can degrade the very gains in soil quality that the system seeks. 'Solving' herbicide resistance by doubling down on chemical reliance, perhaps through stacked transgenic crop cultivars to allow greatly expanded, novel use of an old herbicide, can result in mishaps such as the 2017 'dicamba debacle'. If practitioners are willing to put aside orthodoxies, there are considerable efficiencies to be gained by making judicious use of a variety of tools, as anyone familiar with integrated weed management knows. In Middle Way approaches to ecological intensification of agriculture, all tools are potentially at the manager's disposal. This does not mean, however, that such systems are *laissez faire*. Rather, their design ideally begins with a transdisciplinary group of stakeholders to set benchmarks for various dimensions of system performance (e.g. ecological, economic, human health, social, etc.) and uses these to guide management decisions. In a proof of concept Middle Way production system in Boone, Iowa, USA, cropping system diversification balanced productivity, profitability and environmental health in comparison to the business as usual model, which favored productivity and profitability at the expense of environmental performance indicators. By their very nature, Middle Way production systems will vary with biophysical environment and cultural preference. With its history of producing weed management efficiencies through integration of complementary tactics, weed science has much to offer the design teams that shape Middle Way systems of the future.

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## **Artificial Intelligence: smart machines for weed control and beyond**

Ben Chostner

Blue River Technology, Sunnyvale, California, United States of America

Emerging technologies such as artificial intelligence, computer vision and robotics are just beginning to be integrated into production agriculture. These technologies promise to enable the next wave of precision agriculture by moving from zone management to plant management. Learn about the opportunities and challenges of managing every plant, and how Blue River Technology is utilizing these technologies to deploy See & Spray machines that apply herbicide only to weeds. By combining the latest in sensors, processors and deep learning neural networks it is possible to classify each plant in the field as crop or weed with high accuracy, and do so while traveling through the field at 10-15 kph. A custom spray system then delivers a dose of herbicide precisely to the weed. This approach uses up to 90% less herbicide than broadcast applications, and through application only to the weeds also enable the use of non-selective herbicides not previously available for use in post-emergent situations.

## **Tackling global crop production challenges for sustainable food and nutrition security**

Martin Kropff

International Maize and Wheat Improvement Center, Netherlands

Since the 2008 food price crisis, agri-food systems have become even more central to global challenges such as the ones related to population growth, climate change and environmental protection. These challenges have been well defined in the United Nation's Sustainable Development Goals. In spite of the global effort to address these challenges we need to intensify our effort to achieve key goals such as zero hunger and poverty reduction. Agri-food systems, especially in the developing world, are fragile and vulnerable to shock while we have to produce more nutritious food with less resources and it has to be better. In some areas in the world the use of water for crop production is not sustainable.

Our ability to tackle global crop production challenges requires thinking beyond production and productivity. Multiple converging food production challenges threaten our collective ability to feed more people in increasingly more fragile environments. Understanding the severity of these challenges and the action needed to tackle them is high on the international research agenda but research must be informed by and respond to real problems and challenges farmers are facing.

Biotic and abiotic pressure on farming systems such as weeds, pests and diseases, drought/flooding and heat stress are increasingly challenging. These are exacerbated by socio-economic factors such as price and market volatility. Most of the extra 2 billion people expected by 2050 will be born in Africa and Asia - in countries already pressured by large populations & food-nutrition security problems.

Tackling global crop production challenges is critical to feeding our growing global population. Doing this requires adopting a systems approach - using both life and social sciences to explore solutions across the value chain. Increasing crop production requires both optimally using existing tools while also developing new technologies. Breeding can be sped up with the use of molecular tools including gene editing. Mechanization is a crucial approach in developing countries where an agricultural transformation is needed. Information and communication technologies have the potential to help farmers to make better production (efficient use of water and nutrients, disease and pest management) and marketing decisions but deployment of such technologies in smallholder farming systems is still in its infancy. And, last but not least, crop protection should be cheap and effective while protecting the environment as much as possible.

In this presentation the opportunities for science and technology in tackling holistically the global crop production challenges by innovation are discussed with special reference to the role of international agricultural research.

**S01-O Student Oral Session**  
**ORAL PRESENTATIONS**

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## **Upward N redistribution- Post-harvest effect of catch crops on creeping thistle (*Cirsium arvense*)**

Thi Thuy Hong Phan

Section of Crop Science, TAASTRUP, Denmark

Weed control is important in organic farming to improve crop yield. In Northern European countries, the need for mechanical weed control restricts the use of green manure-based cover crops, a key resource of N supply. In a three-year experiment repeated in three seasons, we aim to compare the effect of cover crops and autumn tillage on the N uptake and N competition of barley and thistles growing in the following year. In the autumn,  $^{15}\text{NO}_3^-$  tracer was applied to the topsoil and made subject to leaching or cover crop uptake.  $^{15}\text{NO}_3^-$  content in the soil profile was measured in the following spring, followed by measurement of  $^{15}\text{N}$  uptake by barley and thistles during crop growth. Cover crops reduced leaching of the residual  $^{15}\text{NO}_3^-$  from the topsoil to the deeper soil layer (1.0-1.5 m) by a factor of >18 compared to the tillage treatment. A significant effect of cover crops on soil N distribution was observed: 67 - 85% of the total recovered  $^{15}\text{NO}_3^-$  was found in the upper 0.5 m under the cover crop treatments, while in the tillage treatment only 4-27% of the recovered  $^{15}\text{NO}_3^-$  were found in this upper soil layer. By keeping the  $^{15}\text{NO}_3^-$  in the topsoil, cover crops shifted  $^{15}\text{N}$  availability away from the deep-rooted thistles and towards the shallow rooted spring barley, and strengthened the competition of barley against thistles. In contrast, the deep N redistribution following the autumn tillage practice enhanced N uptake of thistles. Therefore, intensive autumn tillage used as a tool of perennial weed control should be removed or reduced and cover crops can be part of the integrated perennial weed management in organic cereal production.

## Phytotoxic metabolites with potential herbicidal activity produced by pathogenic fungi of buffelgrass and cheatgrass

Marco Masi<sup>1</sup>, Susan E. Meyer<sup>2</sup>, Clement Suzette<sup>2</sup>, Massimo Cristofaro<sup>3</sup>, Alessio Cimmino<sup>1</sup>, Antonio Evidente<sup>1</sup>

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Cheatgrass (*Bromus tectorum*) and buffelgrass (*Cenchrus ciliaris* or *Pennisetum ciliare*) are two grasses native to the Old World which have become highly invasive in semiarid ecosystems of western North America. Both weeds are negatively influencing the native vegetation and are associated with increased fire frequency. To obtain potentially safe bioherbicides to use against these invasive weeds, fungal pathogens were isolated from populations in the introduced North American range. These included *Pyrenophora semeniperda* from cheatgrass and *Pyricularia grisea* and *Cochliobolus australiensis* from buffelgrass. The ability of these pathogens to produce phytotoxic metabolites *in vitro* was then evaluated. *P. semeniperda* produced large quantities of cytochalasin B along with some related compounds, the new pyrenophoric acid and pyrenophoric acids B and C, the known abscisic acid and several spirocyclic lactams including the new spirostaphylotrichin W. From the organic extract of *P. grisea* two monosubstituted hex-4-ene-2,3-diols, named pyriculins A and B, (10S,11S)-(-)-epipyriculol, *trans*-3,4-dihydro-3,4,8-trihydroxy-1(2H)-naphthalenone, and (4S)-(+)-isosclerone were isolated. *C. australiensis* produced a new trisubstituted 2,3-dihydropyrano[4,3-*b*]pyran-4,5-dione, named cochliotoxin, two new tetrasubstituted 3-chromanonacrylic acids, named chloromonilinic acids C and D, and the known chloromonilinic acid B, radicinin, radicinol, and their 3-epimers. This communication will illustrate an overview of the work carried out to isolate and chemically characterize these phytotoxins produced in solid and liquid culture. Furthermore, the role of these compounds in the symptoms induced on buffelgrass or cheatgrass as well as their potential use as bioherbicides will be discussed.



## **Living mulch and cover crops enhance weed seed predation after crop harvest**

Matthias Schumacher, Roland Gerhards

University of Hohenheim, STUTTGART, Germany

Weed seed predation interferes with the entry of new weeds into the soil seed bank and substantially reduces their amount. However, crop harvest represents a major habitat disturbance for the seed predators that tremendously constrains their activity. Living mulches and cover crops might be a useful tool in providing a suitable habitat to increase seed consumption after harvest. To test this hypothesis we measured i) weed seed predation rates and ii) Carabid beetle activity-density in fields with either a grass-clover living mulch (LM), a cover crop mixture (CC) or bare soil (BARE). One reference measurement was performed in cereals in July. After the cereal harvest monthly measurements were gathered from August to October using seed cards and pitfall traps. In July seed predators consumed approximately 50% of the offered seeds in all fields. After harvest the LM fields regained a seed predation rate of around 50% during September and October. In CC fields the predation rates decreased to 18% in September, but recovered to 30% by October, while the BARE treatment continuously decreased to 10%. Carabid beetle activity-density in September was 6 to 8 times higher in LM (30 beetles/trap) and CC (40 beetles/trap) fields compared to BARE soil (5 beetles/trap). We conclude that living mulches are able to prolong the activity period of seed predators in the field and maintain high seed predation rates after harvest. Cover crops are also able to enhance seed predation rates, but their effect is restricted to late autumn after they have built up enough biomass to cover the soil. Therefore living mulches and cover crops do not only provide direct biological weed control, but also indirectly enhance ecosystem services like weed seed predation.

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## **Amaranthus palmeri and Amaranthus tuberculatus (rudis) - troublesome weeds in irrigated crops**

Inon Yadid, Zvi Peleg, Baruch Rubin

Hebrew University of Jerusalem, REHOVOT, Israel

*Amaranthus palmeri* and *A. rudis* are summer annual weeds that due to their vigor, prolific seed production and seed longevity in seed bank cause severe damage to irrigated crops. Numerous reports have shown that these weeds evolved resistance to several herbicide mode of actions (MOA). These alien weed species are dioecious enabling them outcross with other *Amaranthus* spp. ALS-resistant *A. palmeri* was reported more than a decade ago, whereas *A. tuberculatus* has recently started to infest irrigated crops in Israel. The objectives of this research are: To study the biology and the distribution of *A. tuberculatus* in ruderal and segetal habitats; response to herbicide as compared to *A. palmeri*, and possible outcrossing between the two species. More than 20 populations of *A. tuberculatus* were collected throughout the country and mapped (GIS), both in ruderal and segetal habitats. Majority of populations were found in proximity of animal feed production and fishponds, indicating possible entry to the country with imported grains. Most of the ruderal populations were susceptible to the tested herbicides, whereas those collected in irrigated crops adjacent to *A. palmeri* survived high rates of ALS herbicides, indicating possible outcrossing between the species. Studies on the extend of outcrossing between the two species under different climatic conditions is in progress. The level of *A. tuberculatus* distribution, growth and propagation characteristics found so far imply that special measures should be taken in order to prevent the potential threat of its future infestation in cultivated fields.

## **Distribution, invasiveness and morphological characterisation of weedy sunflower in Republic of Serbia**

Darko Stojićević<sup>1</sup>, Dragana Božić<sup>2</sup>, Sava Vrbničanin<sup>2</sup>

<sup>1</sup>Higher Education Technical School of Applied Sciences, POŽAREVAC, Serbia

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Weedy sunflower is invasive weed on the territory of the Republic of Serbia whose expansion has been significant in recent years. It is formed of volunteer plants of common sunflower (*Helianthus annuus*) which persist for a long period of time on one field, where they cross with other forms of sunflower and become more aggressive. Weedy sunflower is very present in agricultural fields and involves big loses of yield (5-35%). On the territory of the Republic of Serbia weedy sunflower is mostly present in Vojvodina province and in small percent on other sites where farmers are growing sunflower. Distribution and invasiveness of weedy sunflower and mapping of sites with that species were the aim of this study. Presence of weedy sunflower was indentified on almost 200 localities. Population size was in the range from 25 to 2 000 000 plants, with density on some sites even to 20 plants/m<sup>2</sup>. Collected data is used for making UTM map of distribution of weedy sunflower. Volunteer plants are also traced because they are a source for more aggressive variety like weedy sunflower. Also, 13 morphological parameters were monitored in 170 plants of weedy sunflower, 50 plants of hybrid sunflower (*Helianthus annuus*) and 30 plants of jerusalem arthichoke (*Helianthus tuberosus*). Statistically significant differences between the tested weedy sunflower plants, hybrid sunflower and jerusalem arthichoke were observed. Increasing of agricultural areas with hybrids of sunflower tolerant to herbicides (ALS inhibitors) involve increasing of risk of potential gene flow between tolerant hybrids and weedy sunflower. In recent years on agricultural fields, there is an increasingly frequent occurrence of resistant populations, and for this reason monitoring of distribution and invasiveness of this species is very important.

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## **Factors influencing the composition of the weed flora of arable lands from Central Romania**

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Weed species loss due to intensive agricultural land use has raised the need to understand how traditional cropland management has sustained a diverse weed flora. We evaluated to what extent cultivation practices and environmental conditions affect the weed species composition of a small-scale farmland mosaic in Central Transylvania (Romania). We recorded the abundance of weed species and 28 environmental, management and site context variables in 299 fields of maize, cereal and stubble. Using redundancy analysis, we revealed 22 variables with significant net effects, which explained 19.2% of the total variation in species composition. Cropland type had the most pronounced effect on weed composition with a clear distinction between cereal crops, cereal stubble and maize crops. Beyond these differences, the environmental context of croplands was a major driver of weed composition, with significant effects of geographic position, altitude, soil parameters (soil pH, texture, salt and humus content,  $\text{CaCO}_3$ ,  $\text{P}_2\text{O}_5$ ,  $\text{K}_2\text{O}$ , Na and Mg), as well as plot location (edge vs. core position) and surrounding habitat types (arable field, road margin, meadow, fallow, ditch). Performing a variation partitioning for the cropland types one by one, the environmental variables explained most of the variance compared with within crop management. In contrast, when all sites were combined across different cropland types, the crop-specific factors were more important in explaining variance in weed community composition.

### **Acknowledgements**

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## Testing the applicability of regression models to estimate weed seed production

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Measuring weed seed production is extremely labor-intensive. Less intensive methods rely on relationships between panicle biomass and seed production, but it is unknown how general these relationships are. In this study, we tested this generality for *Echinochloa crus-galli* (L.) P. Beauv. in three different maize fields in North-eastern Germany. We expected that the existing relationship (Norris, 1992) is not generally applicable.

In three commercial maize fields, six blocks (10.5 x 13.5 m), each with 12 plots (1.5 x 1.5 m) were installed. Half of these plots were enclosed by a 60 cm high plastic frame that prevented access to seed predators. In August 2014, seeds of *E. crus-galli* were manually applied at 300, 600, 1200, 2400 seeds m<sup>-2</sup> in two plots and no seeds (control) in four plots per block. In half of the blocks weeds were allowed to germinate and grow after maize developmental stage BBCH 13. Weeds were not allowed in the other half. Seedlings were divided in four cohorts depending on the time of emergence. As soon as panicles were formed they were wrapped in crisp bags to prevent seed loss. All panicles were weighted and the length measured. For a sample of 178 panicles, the relationship between panicle biomass and number of seeds was determined, using linear regression analyses. Additional explanatory variables were fields, sowing densities, cohorts and weed control intensity.

Results showed that, (i) the relationship between seed number and biomass was field-specific and (ii) seed production was influenced by sowing density in one field (iii) effects of cohorts and weed management intensity were discounted in biomass. Consequently, when estimating weed seed production, a calibration line needs to be established for each field separately and people need to be aware that weed density can influence this relationship.

## Herbicide efficacy estimation of ALS-inhibitors in *Stellaria media* L. and *Papaver rhoeas* L.

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The number of resistant *Stellaria media* L. and *Papaver rhoeas* L. populations to ALS inhibitors is steadily increasing since their first documentation (1988, 1993). Yet, reliable tools for a fast herbicide efficacy estimation in dicotyledonous weeds are still missing. Plant stress measured by the maximum quantum yield of photosystem II (*Fv/Fm*) could help. In this study, (*Fv/Fm*) was used to quantify the herbicide induced stress 7 consecutive days after treatment (DAT) and compared it to the common herbicide efficacy assessment, measured 28 DAT.

One sensitive and one resistant population to ALS inhibitors were used, both from *S. media* and *P. rhoeas*. The trial was carried out as a random complete block design with 3 herbicide treatments, 4 repetitions and 3 plants per repetition at two different fields in southern Germany (Plieningen and Renningen). Pre-grown plants were transplanted into the field. The application of: i) 19.3 g a.i. tribenuron ha<sup>-1</sup>, ii) 8 g a.i. metsulfuron ha<sup>-1</sup> and iii) 5 g a.i. florasulam ha<sup>-1</sup>, were carried out during the 4 to 6 leaf growing stage. Transplanted, unsprayed plants served as the control. On 1-7, 9 and 14 DAT the *Fv/Fm* value of each plant was assessed with a portable PAM fluorometer (WeedPAM, Heinz Walz GmbH, Germany).

The *Fv/Fm* values of sensitive *S. media* plants was decreased by 47, 39 and 44% relative to the untreated control, already 4 DAT while the *Fv/Fm* of the sensitive *P. rhoeas* plants had a 37, 30 and 36% drop towards tribenuron, metsulfuron, and florasulam respectively. At the same time, compared to the control, the resistant plants of both species had a drop of less than 20% in all herbicides. These results show the potential of the *Fv/Fm* values for herbicide stress identification. Moreover, this technology, can be used as a faster predictor towards the herbicide efficacy.

**S02-O Prodiva Project  
ORAL PRESENTATIONS**

## **Crop diversity - a crucial remedy for weed management in organic cropping**

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A wide variety of crop species in crop rotations has turned out an essential means to obtain diversification in organic cropping systems. Moreover, the potential of cover crops to assist the main crop in weed suppression has been studied in Denmark, Finland and Latvia. Long-term field experiments in Denmark and Latvia demonstrated that crops with different life cycles and life spans in crop sequence resulted in a relatively low and manageable pressure of annual weeds. Soil samples taken in Denmark after 20 years of organic cropping showed that when grass-clover was grown as a green manure for 25 % of the time or more, the weed seed bank was more than halved as compared to the rotation without grass-clover. Moreover, for the efficient suppression of perennial weeds, such as *Cirsium arvense* and *Sonchus arvensis*, periods with grass-clover for mowing were required. In contrast, grass-clover rather contributed proliferations of *Elytrigia repens* in Danish experiments. Also legumes and cereal-legume mixtures promoted the growth of *E. repens* and cover crops obstructed the possibility for mechanical post-harvest control. As shown in Finnish experiments, cover crops (clover species and grasses) undersown with spring cereals in early spring, were too slow to effectively hamper the emergence and early growth of annual weed species but later in the growing season they interfered with weeds. Therefore, cover crop termination by tillage should be delayed until late autumn or next spring to benefit from this late suppression. In Latvia, the weed density in grassland, one year after barley with undersown clover-timothy, was significantly lower than without undersowing. Even *E. repens* was suppressed by including undersown red clover, clover mulch and winter rye in crop rotation. The study was part of the PRODIVA project (Core Organic Plus) in which crop diversification for better weed management has been studied.



### **Crop species mixtures for weed suppression**

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The aim of this study is to assess the weed suppressive ability of crop mixtures and impact of weeds on crop yield as a function of species and densities by means of field experiments and controlled experiments with mixtures of barley and pea, conducted in Sweden and Poland. The performance of crop mixtures was compared to the performance of pure crops with regard to their ability to suppress weeds. In the fields, natural weed populations were used while the controlled experiments employed *Elytrigia repens* or *Sinapis alba* as a model weed. Preliminary results from the controlled experiment in Sweden showed that the presence of a crop (sole crop or intercrop) significantly diminished the growth of *E. repens*. The ability to compete was lower in peas, compared to barley and the intercrop. No significant differences were found in the ability to withstand competition between sole cropped pea, sole cropped barley, and the intercrop. In the field experiment, the overall weed pressure was rather high (> 750 g DM m<sup>-2</sup>). No significant differences in total dry matter were found for spring barley, sown in different proportions in pea/spring barley mixtures. Total dry matter of peas was related to pea seeding density. Preliminary results from the field experiment in Poland showed that crops significantly suppressed weeds compared with the treatment containing only weeds. In the crop combination containing sole peas and 70% peas + 30% spring barley, the crop was not able to suppress the weeds as efficient as in treatments with a higher proportion of spring barley. No significant differences in seed yield were found between the treatments. In the glass house experiment with *E. repens*, the rhizome dry weight and rhizome length were significantly lower in treatments with both crop and weeds as compared with pure weeds.

## **Can variety mixtures of spring cereals benefit weed management?**

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Variety mixtures of spring cereals are gaining interest for greater yield stability based on resilience towards climatic conditions and diseases. Cultivars with suppressive abilities against weeds are increasingly used as part of an integrated weed management scheme, but the added benefits of mixing varieties to support weed management is less well understood. As part of the PRODIVA project, mixtures of barley and oat varieties, respectively, were established to study their weed suppressing ability and weed tolerance, including any added benefits compared to single varieties. The hypothesis was that the earlier the ground is covered and the denser the canopy becomes, the higher weed tolerance and suppression are achieved. Due to lack of information on variety characteristics, not all mixtures were based on existing knowledge of characteristics, but were characterised during the experiments.

Field experiments were established in Latvia, Poland and Denmark in either 2 or 3 growing seasons, partly with a surrogate weed and partly with natural weed populations. In each country, 6 barley and 3 oat varieties were sown both as single varieties and in mixtures. The varieties differed among countries. Growth parameters were measured to follow the development through the growing season, *e.g.* height, leaf area index, weed biomass, and crop biomass. Furthermore, yields in terms of quantity and quality were measured.

There were no consistent benefits for weed management of combining the varieties, the results, however, indicate larger potential for barley than oat, and some barley mixtures had more potential than others. The size of the weed population influenced the correlation among the measured parameters.

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**The vegetation of Baltic organic cereal fields as shaped by site and management**

Merel A.J. Hofmeijer, University Rostock, Germany

Weeds remain to be the main challenge within this agricultural system, especially perennials in the north European realm. Because of increased interest in agrobiodiversity functioning, weeds should be kept within manageable limits, while on the other side encourage a specie rich weed flora. One of the PRODIVA project objectives is to investigate to which extent these two aspects can be addressed through the use of diversified crop management. In order to research this objective, weed and management data were obtained from organic farms in the baltic region over the course of two years (2015-2016) in spring sown cereal crops. The impact of the local environment and management factors on the occurring weed communities was studied in multivariate analysis approaches, followed by the separate crop diversity effects. We plan to present results of the analyses of this data.

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**From practice to science and back - knowledge and information chains in PRODIVA**Bärbel Gerowitt

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To organize and manage the dissemination process has become a general task within international projects. Within the project PRODIVA (Crop diversification and weed management) we organized these chains as returning cascades between practice (advisors and farmers) and scientists in the Baltic area. A network of farmers was created in each participating region. We collected and used practical knowledge about dominating weed species in Organic spring cereal production. This data can be compared with monitored weeds in the regions. Together with the monitored weeds management data was collected on-farm. Relating this data to the results of experimentally investigated crop diversification tools, being subsidiary crops in the rotation, crop mixtures and variety mixtures show existing overlaps and gaps. Success stories of farms implementing crop diversification links the results in a way most suitable for direct dissemination from farm-to-farm.

**S03-O Chemical Weed Management  
ORAL PRESENTATIONS**

### **Enhancing sensitivity of *Amaranthus palmeri* to glyphosate with quinate**

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The overuse of the worldwide known herbicide glyphosate have resulted in resistant weeds, such as the evolved resistant populations of *Amaranthus palmeri* due to EPSPS amplification. Glyphosate functions by inhibiting the enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase in the biosynthetic pathway of aromatic amino acids (AAA) (the shikimate pathway) and several physiological effects on this pathway have been reported, such as quinate accumulation. Quinate accumulation after glyphosate raised the question if glyphosate toxicity would be enhanced if it was combined with quinate. The objective of this study was to ascertain if glyphosate efficacy on *A. palmeri* populations (sensitive and glyphosate-resistant) could be enhanced with quinate spray, thus leading to a lower rate of herbicide use. Plants were grown in aerated hydroponic culture under controlled conditions and sprayed with glyphosate (0.25 times recommended field rate in the sensitive population and 0.5 times recommended field rate in the resistant one) and/or quinate 24h after herbicide treatment. After 3 days of glyphosate treatment shikimate, quinate and AAA content was measured in leaves. Visual effects were monitored. In the treatment of quinate alone an increase of the amount of the L-Tyrosine and L- Phenylalanine was detected, suggesting an increase of carbon flux in the post chorismate pathway through the Chorismate Mutase. In both populations a deregulation of the shikimate pathway was detected after glyphosate applied alone and this deregulation was exacerbated when quinate was applied after glyphosate. In sensitive plants, lethality was only detected when quinate and glyphosate were applied together. These results suggest that application of quinate with glyphosate may be used to enhance glyphosate efficacy.

**Predicting the transport and residual activity of the herbicide sulfosulfuron in soil profile**

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Sulfonylurea herbicides are used extensively for weed management in various crops. In neutral-alkaline soils, several sulfonylureas, such as sulfosulfuron, have long-term residual activity, due to slow degradation, that is adversely affected by leaching from the upper soil layer to deeper layers. To optimize application protocols, the co-effects of degradation and transport needs to be considered. Our objectives were to quantify sulfosulfuron degradation rate and its transport in alkaline clayey soils and examine the feasibility of predicting the transport profile with HYDRUS-1D - a software for modeling water flow and solute transport in variably saturated porous media. Samples of three clayey soils (pH 7.6-7.8) from northern Israel were fortified with sulfosulfuron (80 ng g<sup>-1</sup> soil). After incubation (25°C) for different time periods, the herbicide was extracted and subsequently quantified in LCMS/MS. Herbicide transport was investigated in soil columns (8:30 cm, diameter:length) in which sulfosulfuron (40 ng g<sup>-1</sup> soil) was mixed with the top 10 cm. Sulfosulfuron concentration was estimated in six segments of the column (each 5 cm long) using sorghum bioassay, after irrigation with 650 mL (50 mL 10 min<sup>-1</sup>). The same scenario was simulated in HYDRUS-1D, and predicted concentration were compared to the observed. Sulfosulfuron degradation in the soils followed first-order kinetics, with different rates ( $k \pm SE$ ) [0.032 $\pm$ 0.002, 0.028 $\pm$ 0.002, and 0.015 $\pm$ 0.001 days<sup>-1</sup>] that could relate to the extent of residual activity in these soils. In the sorghum bioassay, similar profiles of sulfosulfuron were detected in all tested soils: lower response at the upper three compared to the lower three segments of the column, with average sulfosulfuron concentration of 0.7, 3.5, 7.8, 11.6, 12.5 and 11.1 ng g<sup>-1</sup> soil, respectively. Simulated transport with HYDRUS-1D correlated to the concentrations estimated by the bioassay ( $r=0.95$ ; SE of estimate=1.66), demonstrating the power of this tool for simulating the fate of sulfosulfuron in the soil.

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## **Optimizing herbicide use in cereals - consequences for yield, weed populations and resistance**

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In Germany 50 field trials in winter cereals were conducted between 2012 and 2015. The aim of these trials was to determine the potential for optimize the herbicide use by corresponding to actual weather conditions. There different doses and herbicide programs were applied on ~ 15 sides per year. Herbicide efficacy and yield were measured and weather conditions at and around herbicide application date was observed. Additionally on two sides a permanent trial with fixed herbicide doses in winter wheat was conducted to investigate the shift in herbicide susceptibility as a consequence of repeated application of sublethal doses. This approach was done by comparing of greenhouse dose-response experiment of weed seeds sampled in the field before and after 3 year trial period.

Results showed that without weed control yield was decreased up to 80% depending on present weed species and infestation level. By application of side specific herbicide mixture on average only 50% of registrated dose rate was necessary to ensure yield. However, for high herbicide efficacy (>90%) a dose rate of >65% was necessary. Results of additional permanent trials showed that herbicide resistance level increased very rapidly on field level if sublethal herbicide doses of the same herbicide were applied within three years on the same plots. Correlation of weather conditions and need of optimal side specific herbicide dose was not possible. Reasons for this might be too many possible combination of influencing factors, which can not be described by current data set.



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**Dicamba Volatilization from Field Surfaces In Tennessee , USA**Thomas C. Mueller

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Dicamba-tolerant soybeans have been used in the United States in recent years to improve weed control of glyphosate resistant weeds by the POST application of dicamba. Much interest has been generated by the potential for off-site movement of dicamba, which has resulted in discussions related to the causes of this phenomenon. This report details three field studies, including optimizing field and laboratory conditions to enhance sensitivity and accuracy of dicamba sampling and analysis. Dicamba concentrations were determined using high volume air samplers located inside the sprayed fields followed by lab analysis using LC-MS. The first study showed that diglycolamine (DGA) salt of dicamba was more likely to volatilize from green plant surfaces compared to either tilled bare ground or dead plant material. The second study showed that newer formulations of dicamba had slightly lower volatility under field conditions compared to the diglycolamine salt formulation. The third study indicated no apparent effect on dicamba volatility of adding a commercial formulation of glyphosate to the BAPMA salt of dicamba, although the surface condition of this study was primarily bare soil and as such a different outcome could be postulated based on varying field conditions. All samples showed dicamba above detectable levels, and the typical pattern of dicamba arising from treated plots was correlated to temperature.

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## **Environmental risk assessment of pesticides under revision: Why does this affect smart weed control?**

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Herbicides are key components of the toolbox for smart weed control techniques like precision farming. However, the efficacy of this toolbox depends on the availability of herbicides. In the EU the registration of herbicides as of all pesticides is regulated by Reg. 1107/2009. During the registration process pesticides undergo a thorough evaluation of their efficacy and safety for humans and the environment. The safety assessment is conducted by the European Food Safety Authority (EFSA). Most of the Guidance Documents (GDs) defining the environmental safety standards and risk assessment procedures are under revision. This is also the case for the GD on the risk assessment for non-target terrestrial plants (NTTP). In 2014, EFSA published a scientific opinion providing outlines of aspects considered relevant for the future risk assessment for NTTP, including quantitative proposals for increasing safety factors considerably for the off-field risk assessment. These adjustments would most likely result in a further reduction of registered active substances i.e. herbicides. In addition, a fundamental change of the NTTP definition was stipulated which would include »*plants growing within the fields that are not the intended pesticide target*«. This protection goal is practically not achievable neither with current products nor with current spray techniques. If implemented, the hurdles for (re-) registration would further increase resulting in even less available herbicides. Smart technologies will allow precise and targeted herbicide application in the near future and could mitigate the exposure of NTTP. However, there have to be still different herbicides available to use these technologies in a smart way. We will provide an overview of proposals and perspectives of different stakeholders in the discussions on the NTTP risk assessment revision. We will discuss how these changes may affect standard and smart weed control and we invite weed scientists to take up a stance in these discussions.

**S03-P Chemical Weed Management  
POSTER PRESENTATIONS**

## **Patterns of glyphosate use in North German arable farming**

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Glyphosate is subject of strong controversial discussions, these focuses on two opposing opinions: the over-use of glyphosate in arable farming as well as the importance for the weed management of many farmers. Actually, the European Commission authorizes the use of glyphosate for another five years. Probably, the discussions about glyphosate use in arable farming will be continued within scientific and public communities.

Glyphosate based herbicides are used as pre-sowing, pre-harvest and stubble applications. In this study, we contribute to the discussion with an analysis of glyphosate use patterns in a large-scale 10-year dataset of herbicide use and tillage practices on arable farms. The study is based on herbicide use data from five districts of Northern Germany. Data were collected from 10 conventional farms in each of five administrative districts for the period 2005-2014.

We investigated glyphosate use on field level - different aspects were focused to describe the role of glyphosate in weed management. We analyzed the interplay of tillage practices, glyphosate use and selective herbicide intensities on the same field in one year, using the Treatment Frequency Index (TFI) to quantify herbicide intensities.

We found that selective herbicide intensities for in-crop weed management were independent of glyphosate treatment prior to the crop. In contrast, pre-harvest glyphosate intensities were affected by in-crop selective herbicide intensities. Plough tillage significantly reduced both treatments with glyphosate - pre-crop such as pre-harvest treatments.

The current debate about glyphosate in Europe invites to further interpret our results in the light of possible regulation attempts to reduce glyphosate use, for example a volunteer reduction possibly supported by advice and information.

## Effect of species composition on efficacy of vegetated buffer strips for herbicide runoff mitigation

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Vegetated buffer strips (VBS) may play an important role in creating and maintaining biodiversity, preventing soil erosion and contamination of superficial water bodies from plant protection products (PPPs) and nutrients, as well as in safeguarding the agroecosystem landscape. The aim of the study was to assess the efficacy of VBS with different species composition in reducing concentration of herbicides into the runoff water. Evolution in time of species composition of VBS was also monitored. Two types of VBS were tested: mix of *Festuca arundinacea* and *Trifolium pretense* (mix A) and mixture of leguminous species, including *Trifolium repens*, *T. pratense*, *Medicago sativa*, *Lotus corniculatus*, *Onobrychis viciifolia* and *Hedysarum coronarium* (mix B). Plots with bare soil without covering were included as control (check). Trials were conducted on maize, vineyard and peach orchard fields in four areas of North West Italy during 2015 - 2017. VBS were 5 m wide and were placed downhill of 55 to 200 m<sup>2</sup> plots. Plant growth was assessed periodically and VBS were mowed at 5-8 cm height at *T. repens* flowering. Runoff water were collected after each event and analysed through HPLC or LC-MS/MS analysis. Terbutylazine, S-metolachlor, mesotrione and desethyl-terbutylazine were searched in maize, while glyphosate and AMPA in vineyard/orchard. Very uniform coverage and stable composition was maintained in mix A, while dominance of one to two species (*M. sativa*, in particular) was observed in mix B. In maize, the highest terbutylazine concentration at 78 DAT was given by check plots (0.35µg/L), while lower concentrations were obtained in mix A (0.12µg/L) and mix B (0.21µg/L) plots. Similar trends were observed for desethyl-terbutylazine and S-metolachlor. In peach orchard, concentration of glyphosate at 97 DAT ranged from 0.75µg/L (mix B) to 0.13µg/L (mix A), while concentration of AMPA was always <0.1µg/L.

### **Herbicide adjuvants with multifunctional activity**

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Efficacy of herbicides is reduced by various environmental, biological and application factors. Herbicides to be effective must adequately contact weeds, retain on their surface, be absorbed to plant cells and reach toxic levels at the site of action. Usually only a small fraction of active ingredients are biologically utilized and because of this relatively high rates of herbicides must be applied to provide adequate weed control efficacy. To overcome unfavourable factors responsible for efficacy activator and utility adjuvants are incorporated in the herbicide formulations or added to the spray mixtures. A single-component adjuvants (i.e. surfactant, oil or ammonium ions) rarely optimise spray mixture characteristics and provide enhanced droplets retention, optimum spray deposit formation, absorption of active ingredient and finally biological performance. To optimize herbicide performance under a wide range of conditions liquid multifunctional adjuvant formulations were invented. These adjuvants include modified oils, ammonium fertilizers, organic acids, non-ionic and ionic surfactants, pH buffers and humectants that act additively and/or synergistically in mixtures. In the year 2015 studies on development of new, improved multifunctional adjuvants for herbicides and other pesticides were undertaken. In this studies much attention has been paid to the selection of more environment friendly components of such adjuvant formulations. In laboratory experiments physical and chemical properties of multifunctional adjuvants, quality of spray mixtures, including their pH, dynamic and static surface tension and spray droplet contact angle were assessed. In field experiments new developed adjuvants were tested with different herbicides. Efficacy data indicate that herbicides applied in various conditions with the new tested multifunctional adjuvants performed substantially better than with the standard single-component adjuvant formulations.

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## **Efficiency of Herbicides to Control of Sumatran Fleabane (*Conyza albida*) in Northwest Turkey**

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Sumatran fleabane [*Conyza albida* Willd. ex Spreng. syn. *C. sumatrensis* (Retz.) E. Walker] is a weed that invasive, exotic, and occurring in orchards, vineyards, roadsides and other non-agricultural areas. Herbicides have played important role in the management of sumatran fleabane globally. In the northwest part of Turkey many peach producers have complained that they have had ineffectiveness chemical control on *S. fleabanes* compared with it used to be. An experiment is conducted in controlled conditions with Glyphosate, Chlorsulfuron, and Metribuzin using recommended doses and double doses. A population was not controlled. The other two were controlled but the rate of control was also less than acceptable level. These shows that herbicide resistance most probably has been evolved.

## Effect of fabric covering on efficacy, selectivity and residue activity of pendimethalin in lettuce

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The objective of this study was to compare the effect of nonwoven fabrics used for a growth acceleration in lettuce on the efficacy, selectivity and residual activity of pendimethalin herbicide at varying application rates.

Plot field trials were carried out in lettuce in Prague, Czech Republic in 2011-2013. Pendimethalin (Stomp 400 SC) was used at two application rates (600 and 1,200 g ha<sup>-1</sup>). Pendimethalin was applied shortly before lettuce planting (end of March). After planting (same day), halves of plots were covered by the transparent nonwoven fabric (19 g m<sup>-2</sup>). Pendimethalin residues were determined and quantified using liquid chromatography coupled to tandem quadrupole mass spectrometer (Acquity UPLC –Xevo TQS, Waters).

The highest efficacy was recorded against *Chenopodium album* (96–100%) and *Amaranthus retroflexus* (~90%). Efficacy against *Echinochloa crus-galli* ranged between 85% and 100%, against *Solanum phyalifolium* between 77% and 100%, and against *Mercurialis annua* between 72% and 99%.

Selectivity of pendimethalin to lettuce was lower when intensive precipitation was recorded during the growing season. Lettuce injury (15–27%) was recorded on plots covered by fabric and treated by pendimethalin at a rate of 1,200 g ha<sup>-1</sup>. The effect of pendimethalin on lettuce yield was not significant.

Pendimethalin residue concentrations in lettuce heads were significantly influenced by the pendimethalin application rate and by fabric cover, especially at early growth stages of lettuce. The highest pendimethalin concentration at harvest was determined in lettuce grown on uncovered plots treated by pendimethalin at application rate 1,200 g ha<sup>-1</sup> (7–38 µg kg<sup>-1</sup>). Depending on growing season duration and weather conditions, pendimethalin concentrations in lettuce grown under fabric ranged between 0 and 21 µg kg<sup>-1</sup>.

The use of transparent nonwoven fabric cover can help to reduce application rates of soil herbicides and diminish the risk of herbicide contamination in harvested vegetables.



## Effects of Dicamba Micro-Rates on Sensitive Crops

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There is a concern that the widespread use of dicamba-based herbicides in Dicamba-Tolerant Soybeans (Roundup-Ready 2 Xtend) can result in un-intended drift due to windy conditions in Nebraska (and elsewhere). Therefore, the objective of this preliminary study was to establish some baseline indicators on the injury of potentially sensitive crops (eg. non-dicamba-tolerant soybeans, grapes and tomato) to various rates of dicamba-based herbicide. Preliminary field study was conducted in 2016 as a split-plot design with 6 dicamba rates, 2 application times and 4 replications. Dicamba rates were: 0; 1/10; 1/100; 1/500; 1/1000; 1/1500 of the label rate of commonly known dicamba herbicide (560 g ai/ha). Plots had four rows of each soybean type (conventional, organic, Roundup-Ready, Liberty-Link), and Dicamba-tolerant soybeans (as a check) as well as pot-grown grape plants (2<sup>nd</sup> year of growth) and tomato seedlings. There were two application times of dicamba (eg. V2 (soybean 2<sup>nd</sup> trifoliolate), and R2 (full flower) and corresponding size of grapes and tomato. Preliminary results suggested that all four types of non-Dicamba soybeans were very sensitive to dicamba drift. Based on visual ratings, the organic and conventional soybeans were twice as more sensitive to dicamba than glyphosate-tolerant soybeans when sprayed at V2 stage. For example, organic soybeans exhibited as much as 80% and 40% injury by the 1/10 and 1/100 rates compared to 40% and 20% in glyphosate-tolerant soybean, respectively. The 1/10 rate killed grapes (1<sup>st</sup> timing) and severely injured tomato (80%). However, there was only temporary injury in the 2<sup>nd</sup> timing in grapes. The 1/100 (and 1/1000) rate provided only transient symptoms in grapes and tomato (both timings). This is suggesting that grapes and tomato are not as sensitive to dicamba as the non-dicamba soybeans. Yield losses in non-dicamba-tolerant soybean due to injuries ranged from 10-90%.

## New possibilities of post-emergent weed management in winter oilseed rape

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Halauxifen-methyl (Arylex) is a new active ingredient from the group of pyridine-carboxylic acids. Herbicide Belkar (halauxifen-methyl + picloram, 9.59 + 48 g/L, EC) will be available for the use in winter oilseed rape (OSR) in Central Europe from 2018. The objective of this study was to assess the efficacy of Arylex at different concepts of autumn weed management in OSR.

Four plot field experiments were carried out in winter OSR at two different locations in the Czech Republic within two experimental growing seasons. Herbicide Belkar was tested alone (0.25 L/ha) post-emergence (POST), in tank-mix (TM) combination with Metazamix (metazachlor + picloram + aminopyralid) and as a subsequent application after the pre-emergence (PRE) application of Metazamix and Successor 600 (pethoxamid). POST applications were carried out in the middle of September when the OSR had three true leaves and weeds had 3-6 true leaves.

Herbicide Belkar showed a good efficacy (more than 90 %) on many important weeds in OSR: *Galium aparine*, *Geranium pusillum*, *Papaver rhoeas*, *Descurainia sophia* and *Tripleurospermum inodorum*. A lower efficacy (65, resp. 76 %) was recorded on *Viola arvensis* and *Veronica persica*. POST TM combination of Belkar + Metazamix showed an excellent efficacy (more than 97 %) on most of tested weeds like the subsequent POST application of Belkar after PRE application of Metazamix. *V. persica* was better controlled when Metazamix was used PRE (97 %) while *V. arvensis* was better controlled when this herbicide was used in TM combination (80 %).

Efficacy of the Belkar herbicide was less affected by weather (temperature) and soil conditions compared to the reference products Galera Podzim (aminopyralid + picloram + clopyralid and Butisan Complete (metazachlor + dimethenamid-P + quinmerac). The highest efficacy of the herbicide Belkar was achieved in TM combination with Metazamix or when applied after PRE application of Metazamix.

### **Ionic liquids based on choline and fatty acid as adjuvants for herbicides**

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In sustainable agriculture, a significant feature of plant protection is the use of environmentally safe materials that meet the principles of green chemistry. Such a trend is also currently emerging in the use of adjuvants.

Five bio-ionic liquids (BILs) with choline cations and fatty acid anions derived from pelargonic acid, glycerol tristearate, glycerol trioleate, canola oil and coconut oil were synthesized and applied as spray adjuvants with three sulfonylurea herbicides: metsulfuron-methyl, iodosulfuron-methyl-sodium and tribenuron-methyl. Physicochemical properties, including thermal stability, solubility and surface activity, were determined, and the influence of these BILs on herbicidal efficacy was studied in greenhouse tests using four target weed species: *Chenopodium album* L., *Centaurea cyanus* L., *Papaver rhoeas* L. and *Brassica napus* L. Herbicides were dispersed in water and applied using a moving sprayer with a XR TeeJet 110 02 VP flat-fan nozzle delivering 200 L ha<sup>-1</sup> spray mixture at 200 kPa operating pressure to plants at the four-leaf stage (BBCH 14). Herbicide treatments consisted of: metsulfuron-methyl at 4 g ai ha<sup>-1</sup>, iodosulfuron-methyl-sodium at 7.5 g ai ha<sup>-1</sup> and tribenuron-methyl at 15 g ai ha<sup>-1</sup>. Each herbicide was applied alone, and with each choline-fatty acid anion salt at 0.2%, 0.4%, or 0.8% or with 0.75% v/v Actirob 842 EC.

BILs, particularly those with the oleic anion and anions derived from canola oil and coconut oil, greatly improved herbicidal activity. Addition of BILs to the spray mixture significantly reduced the surface tension and contact angle of spray droplets and increased the area of herbicide deposit on the leaf surface.

The specific bio-ionic liquids (BILs) are viable candidates for a new class of bio-adjuvants for herbicides. These BILs improve postemergence herbicidal activities of the sulfonylurea herbicides to levels similar to that of the methylated seed oil, Actirob 842 EC.

### **Strategies for chemical control of *Cyperus esculentus* in maize**

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The perennial sedge *Cyperus esculentus* is rapidly becoming one of the most troublesome weeds across Europe, due to its high adaptive plasticity, the invasive behaviour and the limited availability of effective control methods. The aim of the study was to compare different *C. esculentus* control strategies in maize based on herbicides currently authorized on this crop. The study was conducted in 2016 at Lodi, North-west of Italy on a field of about 1000 m<sup>2</sup>, with 42 plots of 12.5 m<sup>2</sup> each. Treatments were arranged in completely randomized design with three replications. An untreated area of about 1.5 m<sup>2</sup> was left in each plot. Five weed control strategies were compared: pre-emergence (PRE), pre- f.b. early-post-emergence (PRE f.b. E-POST) pre- f.b. late post-emergence (PRE f.b. L-POST), early post-emergence (E-POST), late post-emergence (L-POST). In particular, 2 different mixtures of herbicides were applied in PRE, 1 mixture in PRE f.b. E-POST, 4 in PRE f.b. L-POST, 1 in E-POST and 6 in L-POST. The effects on weed infestation were assessed on *C. esculentus* and on the other weeds by measuring plant density (plants/m<sup>2</sup>), ground coverage (%) and by visually evaluating overall efficacy on a percentage base. *C. esculentus* infestation was quite uniform on the field, with an average density of 41.7 stems/m<sup>2</sup>.

*C. esculentus* was controlled by more than 80% in all PRE and PRE f.b. E-POST strategies, where the active substance S-metolachlor was applied. In general, POST strategies did not control satisfactorily the *C. esculentus* infestation, with the only exception of the mixture containing the active substance isoxaflutole, tiencarbazono-methyl and halosulfuron-methyl, applied in E-POST (efficacy 92%) and the mixture based on halosulfuron-methyl, applied in L-POST (efficacy 95%).

**Weed control at grapevines (*Vitis vinifera* L.)**

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Weeds compete with grapevines for water, light, nutrients, space, etc. They may interfere with harvest, spray equipment, and other operations. The aim of our trial conducted in 2016 and 2017 on the agricultural lands of village Salmanovo, Bulgaria, is to study the weed control opportunities in grapevines. The grown grape variety is Muscat ottonel. Variants of the trial are: 1. Untreated not earthed up control; 2. Earthed up control; 3. Goal<sup>®</sup> 2E (240 g/l oxyfluorfen) 3000 ml ha<sup>-1</sup>; 4. Stomp<sup>®</sup> Aqua (455 g/l pendimethalin) 4000 ml ha<sup>-1</sup>; 5. Goal<sup>®</sup> 2E + Stomp<sup>®</sup> Aqua 300+400 ml ha<sup>-1</sup>; 6. Pledge<sup>®</sup> 50 WP (500 g/kg flumioxazine) 100 g ha<sup>-1</sup> and 7. Pledge<sup>®</sup> 50 WP 400 g ha<sup>-1</sup> as soil application; 8. Roundup<sup>®</sup> (360 g/l glyphosate +180 g/l surfactant) 4000 ml ha<sup>-1</sup>; 9. Roundup<sup>®</sup> 800 ha<sup>-1</sup>; 10. Roundup<sup>®</sup> 12000 ml ha<sup>-1</sup>; 11. Reglon<sup>®</sup> Forte (150 g/l diquat) 3000 ml ha<sup>-1</sup>; Basta<sup>®</sup> 15 SL (150 g/l glufosinate-ammonium) 3000 ml ha<sup>-1</sup> applied during the vegetation; 13. Roundup<sup>®</sup> and water at a 1:1 concentration imported to the weeds by spreading with Herbicide picker<sup>®</sup>. The efficacy of the studied herbicides is recorded by the 10 score visual scale of EWRS (European Weed Research Society). The existing weeds on the field are *Portulaca oleracea* L., *Lactuca serriola* L., *Setaria viridis* L., *Amaranthus retroflexus* L., *Convolvulus arvensis* L., *Poa annua* L., *Stellaria media* L. and *Conyza canadensis* L. The herbicide treatment with Goal<sup>®</sup> 2E and Stomp<sup>®</sup> Aqua, as well as their combine application shows excellent efficacy against the weeds. The application of Pledge<sup>®</sup> 50 WP 400 g ha<sup>-1</sup> should be performed when there are more stubborn weed species on the field. The application of Basta<sup>®</sup> 15 SL and Reglon<sup>®</sup> Forte showed short-term weed control. The usage of Roundup<sup>®</sup> is very effective weed control practice.

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## **ALS-tolerant varieties as a tool for the improvement of winter oilseed rape cropping systems**

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Quite a lot of pests have to be controlled in winter oilseed rape. Consequently pesticide use is high. However, resistance to fungicides and insecticides occur more and more often. Furthermore, ban of pesticides like neonicotinoides for environmental reasons has reduced the options for pest control. New control options are needed to avoid significant yield losses. One idea is to grow a mixture of oilseed rape and another potentially more pest attractive Brassica species. But of course, than there is a need to control the non-oilseed rape plants, before these plants become a competitor to the oilseed rape. So the questions are, are there plants which can attract the oilseed rape pests like rape flea beetle (*Psylliodes chrysocephalus*) and can ALS-tolerant varieties help to do the vegetation management?

To answer these questions a field trial with ALS tolerant oilseed rape was conducted in three year in Southwestern Germany. Oilseed rape was sown with 15 and 50 cm row distance. At same date also different crops were sown into oilseed rape plots. *Brassica nigra*, *Brassica rapa* and *Lepidium sativum* were used as well as a mixture of *Trifolium alexandrinum* and *Vicia sativa*. A mixture of imizamox and flupyrulfuron was used to control the additional plants.

This herbicide mixture was able to control *Brassica rapa* and *Lepidium sativum*, while *Brassica nigra* was controlled by frost. *Vicia sativa* was not controlled by the herbicide combination and was a competitor in oilseed rape in spring. Oilseed rape yield was significant influenced by all Brassica species used in this experiment but not by the legumes. Flea beetle control effect could not been assessed due to low frequency in all years. However, ALS tolerant varieties offer new opportunities and may be by adaption of the growing system this 'biological' control might be useful in the future.

## **Bioassay for detecting the susceptibility of sugar beet on mesotrione residues in different soils**

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Mesotrione is a 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide that indirectly inhibits carotenoid production in susceptible plants. It is non-persistent herbicide and is therefore presumably not phytotoxic for use in crop rotation. Although defined as non-persistent, it is known that mesotrione's persistence can vary depending on the soil's physicochemical properties. The aim of this study was to determine the phytotoxicity of mesotrione residues in sugar beet grown on two different soil types: (1) hipogley and (2) humofluvisol. Mesotrione was applied on both soils at doses of 0; 0.25; 0.5; 1; 2; 4; 6 and 8 µg a.i. per 200 g of soil. The application was done using a thin layer chromatography sprayer. The growing of sugar beet was conducted in a growth chamber under constant climatic conditions for 3 weeks. Symptoms of phytotoxicity (bleaching) were assessed at 7, 14 and 21 days after application (DAA) by using a 0 to 100 % scale (where 0 = no effect and 100 = plant death). The fresh weight of sugar beet was determined on the 21<sup>st</sup> day, after which the total carotenoid content was determined by spectrophotometry. The highest visually evaluated phytotoxicity on both soils was determined on the 21 DAA. On humofluvisol, damages were noticed already at the lowest dose (0.25 µg a.i. per 200 g soil). The sugar beet plants were completely damaged at 4 µg a.i. on humofluvisol and at 6 µg a.i. on hipogley soil. A correlation in the reduction of fresh weight with the reduction of total carotenoid content was established on both soils. A significantly higher reduction of both parameters already at the lowest dose was established on the humofluvisol.

## Effectiveness of Various Vegetative Filter Strips in Preventing Nicosulfuron Runoff

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Herbicides are commonly used plant protection products to control weeds caused substantial yield losses to the crops. However, the herbicides involved in water resources can reduce water quality and damage to the aquatic ecosystems. To mitigate the herbicides reached to the surface water bodies, Vegetative Filter Strips (VFS) is a cost-effective, effective and environmentally friendly solution. Nicosulfuron, belongs to sulfonyleurea herbicides, is labelled to control broadleaves and grass weeds in Turkish corn fields. Although narrow-leaved plant species are used widely in VFS, any study about using these type of plants with imi-tolerant sunflower species in VFS has not been found in the literature. The contamination potential of nicosulfuron used in maize to the surface water resources by runoff and the efficacy of various types of VFSs (4m wide VFSs consisted of narrow-leaved plant species (NLPS: *Festuca rubra* L.+*Bromus inermis* Leyss.) and narrow-leaved + imi-tolerant plant species) to reduce nicosulfuron runoff were determined in field conditions in 2016 and 2017 at Ankara, Turkey. Artificial runoff was generated 1 and 21 days after treatment (DAT) with 50 mm irrigation water using sprinklers, and then nicosulfuron residues in water samples collected from runoff were investigated. Nicosulfuron level in water samples collected from ditch adjacent to the control parcel (unestablished VFS) 1 and 21 DAT was the highest at the both years. VFS planted only NLPS was prevented nicosulfuron runoff at 85 and 76% 1 DAT compared to the unbuffered parcel in 2016 and 2017, respectively. Effectiveness of this VFS 21 DAT to catch nicosulfuron runoff was lower than 1 DAT. Nicosulfuron levels in runoff water collected from NLPS+imi-tolerant sunflower was relatively high in 2016 compared with the VFS planted only NLPS. The differences between the nicosulfuron levels mentioned above may be caused by meteorological conditions such as wind, rain and temperature.



**The effects of herbicides to fresh and dry mass accumulation in maize lines**

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Maize lines are susceptible to various stress factors, including herbicides. The experiment included three susceptible maize lines (ZPKŠ8/1-161su – L1, ZPP608-2/11112111k – L2 and ZPT165b – L3), tested in the field conditions during 2015 and 2016. Herbicide treatments were: nicosulfuron (H1), mesotrione (H2), tembotrione+isoxadifen-ethyl (H3), applied in BBCH 15-16 of maize lines at recommended doses (RD): H1 – 0.75 L ha<sup>-1</sup> (45 g ha<sup>-1</sup>a.i.), H2 – 1.2 l ha<sup>-1</sup> (756 g ha<sup>-1</sup>a.i.) and H3 – 2 l ha<sup>-1</sup> (840 + 420 g ha<sup>-1</sup>a.i.) and double doses (DD): H1 – 1.5 L ha<sup>-1</sup> (90 g ha<sup>-1</sup>a.i.), H2 – 2.4 l ha<sup>-1</sup> (1512 g ha<sup>-1</sup>a.i.) and H3 – 4 l ha<sup>-1</sup> (1680 + 840 g ha<sup>-1</sup>a.i.), and control was without herbicide application. Inbreed lines leaves were sampled 2 days (phase I), 7 days (phase II) and 21 days (phase III) after herbicide application. Fresh mass (FM) and dry mass (DM), after drying at 60 °C were measured.

Higher fluctuations of FM in phase III, as well as DM in all three phases were present in 2016, in comparison to 2015. Phase I was characterised with slightly higher average FM in RD treatment, but higher FM in DD treatments for all herbicides, compared to control. In phase II, lines L2 and L3 had the lowest FM in H3 treatment. Nevertheless, H3 in DD expressed potentially stimulative effect, increasing FM values for L2 and L3 in RD and for L1 in DD. DD treatments were characterised with higher DM concentration for all three genotypes, compared to RD. Present data indicate that meteorological factors highly affect herbicide effect, elevating toxicity, even in phase I. However, up to the 21<sup>th</sup> day after application H3 expressed even stimulative effect by increasing FM and decreasing DM concentration in maize leaves.

## **Influence of adjuvants and pH adjusters on efficacy of metsulfuron methyl**

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Herbicide action is determined by its chemical and physical properties such as surface tension. Adjuvants and pH adjuster added to the spray solution can change physical properties and increase the herbicide effect. The frequent use of ALS herbicides carry the risk of selecting herbicide-resistant weed biotypes which increase the cost of effective weed control. The important topic is optimize weed management systems by addition the most effective adjuvants. The aim of this research was to determine the influence of different additives on the efficacy of ALS inhibitor metsulfuron methyl. The greenhouse experiments were conducted using metsulfuron methyl applied post-emergence at reduced rate (1 g/ha active ingredient) to volunteer oilseed rape (*Brassica napus* L.) as tested plant. Tank mixes were prepared with pH adjusters: citric acids; acetic acid (adjust pH 4); ammonium hydroxide; triethylamine (adjust pH 9) and with adjuvants: methylated rape seed oil; ethoxylated alcohol surfactant and organosilicone surfactant. Additional treatment was also sprayed with recommended rate alone (2 g/ha) and control not treated with herbicides. Visual assessment and reduction of biomass of tested plants was determined as herbicide efficacy. The parameter studied in the laboratory was surface tension for all tanks-mix combinations. A drop of solution was used to measure the surface tension with optical tensiometer (Theta Lite) on a clean and dry Parafilm® surface. The largest decrease of dynamic surface tension did not cause the best efficacy. Results indicated significant efficacy and reduction of volunteer oilseed rape (*Brassica napus* L.) biomass if metsulfuron methyl was applied with citric acid and methylated rape seed oil.

## **Growth stage sensitivity of *Echinochloa crus-galli* to decreasing rates of topramezone and adjuvants**

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Integrated Weed Management requires low herbicide input with post-emergence (POST) application as well as environmentally and economically uses of herbicides. For an effective weed control it is fundamental to set proper time for POST herbicide application when weeds are still at more sensitive phenological stage. Although the best efficacy is expected when herbicides are applied at early growth stages, in the field conditions very rarely plants are at the same stage of development. So the aim of this glasshouse experiment was to determine the efficacy of recommended (67.2 g. a. i. ha<sup>-1</sup>) and reduced dose (44.8, 33.6, 22.4, 16.8 and 8.4 g. a. i. ha<sup>-1</sup>) of topramezone combined with adjuvants (NIS and MSO) on *Echinochloa crus-galli* [L.] P. Beauv. (barnyardgrass) at two different growth stages: three true leaves (BBCH 13) and one-two tiller stage (BBCH 21-22). Topramezone and adjuvants were applied using a precision bench sprayer with a boom equipped with three flat-fan (extended range) hydraulic nozzles with dose spray volume of 300 L ha<sup>-1</sup>, pressure of 215 kPa, speed of 0.75 m s<sup>-1</sup> and using TeeJet<sup>As</sup> nozzles XR11002-VK. The results showed insufficient efficacy of topramezone applied alone in recommended dose on both growth stages (>50%). Applied with adjuvants (MSO or NIS) at BBCH 13 topramezone achieved 100% visually assessed damage and more than 90% biomass reduction, at each applied doses. In contrast, at BBCH 21-22 only double dose of topramezone achieved 73% visual estimated damages. Reduced topramezone doses applied with adjuvants gave insufficient topramezone control at tillering stage (< 40%). Results indicate possibility of reducing dose of topramezone to more than 87% with adding adjuvants (MSO or NIS) but only at early growth stage of barnyardgrass (BBCH 13). Still, at BBCH 21-22 satisfying barnyardgrass control cannot be achieved neither by using topramezone alone nor applied with adjuvants.

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## **Efficacy assessment of five herbicides on dwarf mesquite [*Prosopis farcta* (Banks & Sol.) Macbride]**

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Dwarf mesquite [*Prosopis farcta* (Banks & Sol.) Macbride] is among the most common weed species generally distributed in non-irrigated crops growing in the Eastern Mediterranean region of Turkey. In July 2017, a field experiment was conducted in non-crop site at Çukurova University Agricultural Research Farm to assess the effectiveness of five different herbicides namely, glyphosate diammonium salt, glyphosate potassium salt, glufosinate ammonium salt, 2,4-D amine and 2,4-D + picloram on dwarf mesquite. The experiment was laid out in randomized complete block design with individual plot size of 20 m<sup>2</sup> in four replications. The treatments consisted of five herbicides, a weed-free and a weedy check. The herbicides were applied at three doses, (i) label recommended dose, (ii) two-third dose and (iii) half dose. Herbicide application was done on mesquite at BBCH 33 phenological growth stage, in a spray volume of 300 l water /ha using a motorized backpack sprayer and Teejet nozzle adjusted at a pressure of 3 bar to minimize wind drift. Visual observations of phytotoxic effects on dwarf mesquite were recorded at 1, 3, 5, 7, 14, 21, 28, and 56 days after herbicide application and expressed as percent symptom. Plant height was also recorded. At the end of the experiment plants were harvested and fresh and dry weights of shoot were measured and analyzed. As a result, mesquite control achieved by 2,4-D amine + Picloram, glufosinate ammonium and glyphosate diammonium in both recommended and two-third doses during the first two weeks after treatment was 98%, 88% and 80% respectively. The effect was more pronounced on the plant height and the fresh and dry weights. In addition, herbicides were evaluated against the other weed species found in the study area. Eventually, affective and economic recommendations were suggested for controlling dwarf mesquite in non-irrigated lands.

## Comparative assessment of *Chenopodium album* and *Abutilon theophrasti* response to mesotrione

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The response of *Abutilon theophrasti* and *Chenopodium album* to mesotrione was studied in controlled environment based on plant dry weight, chlorophyll fluorescence parameters ( $F_v/F_m$  and yield  $\Phi_{PSII}$ ) and relative chlorophyll content (SPAD readings). Mesotrione was applied at rates of 3.75, 7.5, 15, 30, 60 and 120 g ai ha<sup>-1</sup> and non-treated plants were served as control. In all treatments herbicide was applied with addition of adjuvant at recommended dose rate. Treatments were applied on the plants of *A. theophrasti* and *Ch. album* at the growth stage of 2 and 4 true leaves, respectively. Herbicides were applied using a laboratory sprayer equipped with an 8001E even spray flat-fan nozzle delivering 200 L ha<sup>-1</sup> at 276 kPa. Chlorophyll fluorescence parameters and relative chlorophyll content were measured 3 days after application (DAA), while for dry weight determination plants were cut 14 DAA and dried 7 days on room temperature. Obtained ED<sub>50</sub>, ED<sub>90</sub> and ED<sub>95</sub> values showed that studied weeds could be effectively controlled with reduced doses of mesotrione at early growth stage of development. ED<sub>95</sub> values estimated for both species for dry weight and chlorophyll fluorescence parameters were lower (22-84 g ai ha<sup>-1</sup>) than recommended dose rate (120 g ai ha<sup>-1</sup>). ED<sub>95</sub> values of SPAD reading for both species were above recommended dose rate with more than 188 g ai ha<sup>-1</sup> needed to reduce 95% relative chlorophyll content. Therefore, chlorophyll fluorescence parameters and dry weight are suitable parameters for estimation effects of low doses of mesotrione on weed species, while SPAD readings were less sensitive and not suitable for monitoring dose response to this herbicide.

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## Troublesome weeds control in peanuts in Israel

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Weed control in peanuts is based on several herbicides which provide a wide spectrum of weed control. Nonetheless, most of these herbicides are old molecules that do not handle troublesome weeds. Preliminary studies have found imazamox to be effective when applied on small caltrops as pre-emergence (PRE), post-emergence (POST), and pre-planting incorporation (PPI) at a rate of 32 ml a.i. ha<sup>-1</sup>. The objectives of this study were a) to evaluate the efficacy of imazamox applied PRE in peanuts and to evaluate its selectivity when applied at five rates between 0-64 ml a.i. ha<sup>-1</sup>; and b) to evaluate the impact of formalin applied as soil disinfection on imazamox efficacy and selectivity to peanuts. Experiments were conducted during 2013 and 2014 in the Negev located in the south part of Israel and in Heffer Valley located near the central Mediterranean coast. The control efficacy and peanuts selectivity to the herbicides examined in this study were compared to the commercial treatment of a tank-mix of terbutryn with S-metolachlor at rates of 500 ml a.i. ha<sup>-1</sup> + 915 ml a.i. ha<sup>-1</sup> respectively. The following herbicides were examined: imazamox 16-64 ml a.i. ha<sup>-1</sup>, bentazon 1440 ml a.i. ha<sup>-1</sup> with pyraflufen-ethyl 10 ml a.i. ha<sup>-1</sup>, imazapic 48 ml a.i. ha<sup>-1</sup>, flumioxazin 75 ml a.i. ha<sup>-1</sup> and aclonifen 1800 ml a.i. ha<sup>-1</sup>. It was found that imazamox applied PRE at 16 ml a.i. ha<sup>-1</sup> and 32 ml a.i. ha<sup>-1</sup> with and without formalin did not injure peanuts. However, the application of imazamox PRE at 48 ml a.i. ha<sup>-1</sup> and 64 ml a.i. ha<sup>-1</sup> resulted in a significant injury and yield reduction compared to the non-treated control. Imazapic and aclonifen effectively controlled *Ipomea* spp. and purple nutsedge. All herbicides were selective to peanuts except the 1800 ml a.i. ha<sup>-1</sup> aclonifen applied POST.

**Sunflower broomrape occurrence under the use of Clearfield ExpressSun and technologies**

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Weeds and *Orobanche cumana* Wallr. pose a major problem in sunflower (*Helianthus annuus* L.) production. The developments of herbicide-resistant crops (HRCs) have provided attractive options for post weed management within the recent years and hence the HRCs are dominating agricultural production systems of sunflower in Greece. ExpressSun® and especially Clearfield® have become the main technologies in sunflower production. The aims of this study were to identify, a) the changes in broomrape abundance in sunflower under the repeated use of the aforementioned technologies last decade and b) the environmental and farming factors which determine broomrape occurrence. During two cultivation periods between July 2012 and July 2015 extensive surveys were conducted in 27 and 50 sunflower fields, respectively, across the Evros region in North - East Greece. In each field we sampled 10 quadrats of 1 m<sup>2</sup> following a Z pattern. *O. cumana* and weed species in each quadrat were counted and their Abundance Index (AI) was determined. Canonical Correspondence Analysis served to quantify the relative contribution of several variables of environment, site and crop management to *O. cumana* occurrence and weed population composition. During the 2012 surveys, broomrape was detected at the 26% of the surveyed fields (frequency of occurrence) and at mean field density (0.77 plants m<sup>-2</sup>). The Abundance Index (36.7), ranked broomrape 13<sup>th</sup> among the 15 most important weed specie. Broomrape's AI (41.2) was slightly increased in 2015, mainly due to its higher mean field density (3.18) and frequency (28%), whereas its uniformity remained the same. Considerable differences in weed species composition of sunflower fields were associated with N fertilization, irrigation, soil organic and clay content and time of sowing. Sunflower broomrape occurrence was mainly associated with rainfed fields, high soil organic and clay content, lower soil pH values and low nitrogen inputs.

This study sponsored by BASF Hellas

**S04-O New Technologies**  
**ORAL PRESENTATIONS**



## **Weed control with autonomous weed mapper in winter cereals**

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The map-based precision weed-control relies on the information from georeferenced weed sampling plots. Researching the literature, we have not found off-line weed-mapping methods, developed for precision weed control technology. The traditional way of weed-mapping is very exhausting, the information transfer, software and algorithm for automatic processing control are not existing and are not resolved. Our goal was to develop a practical algorithm for off-line precision weed control.

Early in the process the autonomous weed mapping robot is working in the field (divided in 18x18 m cells), according to its pre-programmed route and collects pictures in the center of the 18x18 m cells. The pictures are transferred via cloud-based information transmission system to the expert's (integrated pest control) computer. The photo-interpretation and evaluation are done by the expert based on a developed algorithm. Finally, the spraying maps (electronic instructions) are generated and can be transferred to the sprayer's console.

In the spring of 2017 we have been tested the system in several areas, mainly in winter barley. The barley was in good condition, without seeding errors and the experimental area was virtually weed-free. We have found only 5 weed species, *Matricaria inodora* (*Tripleospermum inodorum*) occurred most often and had the biggest coverage. The winter barley experiment was performed on 10.90 hectares and the weed mapper collected 326 photographs. After evaluating the pictures, we have visualized the management zones. According to our results, only 59 cells of 326 (1.9 hectares) required chemical weed control, resulting in 82% herbicide savings.

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## **Using exhaust gas from a combine harvester to kill harvested weed seeds**

Christian Andreasen, Jakob A. Jensen, Klaus Jakobsen  
University of Copenhagen, TAASTRUP, Denmark

Unwanted side-effects of herbicides and increasing problems with herbicide-resistant weeds call for alternative methods to reduce weed infestation on arable land. We investigated if exhaust gas from the combine harvester can be used to harm or kill weed seeds during the harvesting process. The aim is to avoid that harvested viable weed seeds are added to the soil seed bank and become a problem in future growing seasons. A combine harvester cuts and threshes both weeds and the crop. Weed seeds and chaff is separated from the crop seeds during the threshing and cleaning process in the combine harvester, and therefore it is possible to heat the weed seeds with exhaust gas after the separation. Seeds of common weed species were treated with exhaust gas with temperatures of 75°C, 110°C and 140°C for 2, 4, and 6 seconds, respectively. Afterwards, the seeds were germinated for 16 days. We found that 75°C was insufficient to harm the seeds at all three durations, while 140°C for 2 seconds repressed germination of all species. Seed treatments with 110°C gave varying results depending on the duration time and the weed species. Our results showed that there is a potential in developing combine harvesters which exploit the exhaust gas to kill or reduce vigour of weed seeds before they are returned to the field with the chaff.

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## **From image acquisition with unmanned aerial vehicles (UAV) to patch spraying in farmer's fields**

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In 2017, two commercial spring barley fields were patch sprayed based on application maps generated from UAV images. All steps from flight planning, image acquisition, stitching of images, geo-correction of ortho-mosacs, weed detection, and generation and use of application maps are briefly presented and discussed. The accuracy of weed detection and geo-location of weeds and sprayed patches is given in addition to approximate amount of time spent on each job assignment. The study focuses on *Cirsium arvense* (thistle), because it is a common and highly competitive weed, and because it are relatively easy to detect from aerial images in pre-harvest cereals. Furthermore, thistles are distributed in patches, sprayed with glyphosate before or after crop harvest, and controllable with mechanical methods after crop harvest. Thistle patches are also rather stable from one year to the next, which allows the use of maps in the following year. The case-study demonstrates that patch spraying based on UAV images is technically feasible. This is, however, not enough to guarantee implementation in future weed management. Barriers such as time-consumption and user-friendliness related to image acquisition and data processing are discussed, and suggestions are given to future research and innovation that may facilitate the practical application of patch spraying based on UAV imagery.

### **Acknowledgements**

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### **3D Morphological Crop Analysis - A New Methodology for Detection of Broomrape Parasitism**

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Broomrapes are root parasitic weeds that cause severe damage to dicotyledonous crops worldwide. Herbicide application on a field scale can effectively control them, but increasing public concern over potential non-target organism and environmental pollutions motivates adoption of site specific weed management practices. The main challenge in developing such control lies in the fact that most of the broomrapes life-cycle occurs underneath the soil surface, and by the time that their shoots emerge, the damage for the crop has already been done and is unpreventable. In that respect, detection of their early impact in a pre-emergent stage is vital, and so far, no sensing method has proven able to detect them under uncontrolled conditions. In this study we show interaction between broomrape parasitism and the host's morphology, and demonstrate how 3D growth parameters (extracted via 3-D sensing techniques) of the crop can indicate broomrape infection. Additionally, we demonstrated how application of cutting-edge 3-D segmentation methodology allowed estimation of organ-level parameters which were more affected by the broomrape infection. All 3-D morphological parameters evaluated in this study from the host plant exhibited differences between the infected and control plants. However, first internode length was the most sensitive parameter, and together with height was the first to indicate on broomrape infection in a distinct manner. Furthermore, changes in this parameter were detectable early enough for herbicide pre-emergence application. In contrast, 2-D related parameters failed to show infection symptoms. Our 3D morphological modeling is based on commercially available sensors and offers continuous nondestructive morphological evaluations under field conditions; it can promote site specific management and sustainable solutions for root-parasitic weeds and reduce the amount of herbicide used for their control.

### **Available options for remote and ground weed mapping**

César Fernández-Quintanilla, José Dorado, José Manuel Pena, Dionisio Andújar, Francisca López-Granados  
CSIC, MADRID, Spain

A relatively large variety of platforms, cameras, sensors and image analysis procedures are available to map weed presence/abundance at various times and spatial scales. Each of these tools may be particularly well fitted to different conditions and tasks. Remote sensing from satellites or aircrafts can provide accurate weed maps when the images are obtained at late weed phenological stages. For the purpose of using satellite/aircraft images in the early season, more refined imagery acquisition with higher spatial and/or spectral resolution and more sophisticated analyses are required. Cameras located on UAVs have been shown to be adequate for early season weed detection in a variety of wide-row crops. However, early weed mapping in winter cereals is still difficult due to the very small distance between rows and to the quick growth of these crops in the early growth stages. Current techniques for weed mapping from on-ground platforms can achieve this objective using a great variety of non-imaging and imaging technologies. Non-imaging techniques include spectral reflectance, ultrasonic and LiDAR sensors. Weed detection with the aid of imaging sensors is the most investigated technique. Relatively low cost bi-spectral or RGB cameras can acquire images with a high resolution, allowing the identification of plant species based on their location, shape, colour and texture features. Selected case studies will be presented to discuss the opportunities and limitations of each methodology. The suitability of a given platform, camera or sensor for weed mapping depends on various factors: crop/weed characteristics, spectral and spatial resolution, technological cost, etc. The optimal option depends on the specific mapping objectives: regional weed surveys, farm planning, field scouting for identification of risk zones, immediate weed actions (mechanical or chemical), studying weed populations at various spatial scales (farm, field, patch).

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## **Distinguish between bad grass and agricultural cultivation by marking the growth of anthocyanins**

Jackline Abu Nassar, Radi Aly  
Navi y'aar, RAMAT YESHAI, Israel

Our study offers a new technological development, which is also considered to be environmental; the study is based on the distinction between weeds and relevant plants. The distinction will include application of both genetic engineering and precise agriculture in marking plant growth.

The idea tested in this study would be transforming the binary plasmid which consists of the gene myBA, in order to mark the model plant with the color purple; this action will take place under the supervision of the 35S promoter using Agro bacterium. This gene causes an increased production of anthocyanins and an application of the color purple to foliage. Using both sensory devices within the sphere of visible light and calculating the color green indicator herbal index, is supposed to make it possible to recognize and isolate the dyed model plant from the wild ones. Our research results with plants containing various levels of anthocyanins (either all or partially dyed with dark purple).

With the help of PCR and RT-PCR, we were able to prove integration of the gene and to quantify the level of its expression among transgenic plants. By examining the anthocyanins level from a transgenic tobacco leaf extract at a wavelength of 435-635 nm, we were able to observe a higher concentration level than the one of the control group. The resulting difference should provide help for a reliable evaluation of the plagued weeds, which can function as a basis for either taking actions towards extermination or to a future plan to exterminate.

Keywords: weed, anthocyanins, mybA, Precise Agriculture.

## Controlling the spread of invasive alien plants along roadsides with innovative methods

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Roads play a significant role in facilitating the spread of Invasive Alien Plant Species (IAPs) by serving as corridors for movement as well as providing habitat for establishment. However, their occurrence in these highly disturbed habitats is perhaps less of a concern than where the corridor pathway facilitates the spread of IAPs into natural or economically important areas. Therefore, applied strategies to manage this invasion pathway are needed.

In this respect, the project »Controlinroad« (<http://www.controlinroad.org>) was launched in 2017. The aim of this research project is to review the occurrence of IAPs along roadsides, review current control methods and regulations throughout Europe, and to test and evaluate innovative control methods.

Firstly, a survey of IAPs frequently associated with roads was carried out in seven European countries (Austria, Germany, Ireland, the Netherlands, Norway, Sweden, and Slovenia). Based on national lists of IAPs, a literature review and expert consultation, a database of problem IAPs for each country was created. The most problematic species identified include: *Fallopia japonica*, *Fallopia sachalinensis*, *Heraclium mantegazzianum*, *Impatiens glandulifera*, *Solidago canadensis* and *Solidago gigantea*.

The next phase will focus on the application of new innovative control methods. It includes the use of native seed mixtures in combination with beneficial plant microbes to provide rapid ground cover to prevent the re-establishment of IAPs where road construction and/or maintenance activities may have resulted in ground disturbance. The application of high frequency high voltage electrical power to control IAPs will also be tested. All proposed control methods will be assessed using a cost-benefit-analysis for their applicability. In addition, existing legislation, guidelines and best practices in road construction and maintenance for the control of IAPs will be explored based on an extensive literature review and expert interviews.

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### **Rapid diagnosis of herbicide resistance using spectral image analysis**

Do-Soon Kim, Tae-Kyung Noh, Tae-Young Lee  
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Rapid diagnosis of herbicide resistance (HR) is a first step of HR management. Various rapid diagnostic methods have been developed but most of them are destructive and require significant time and effort for diagnosing HR. As plants reflect unique spectra responding to herbicide treatments depending on herbicide modes of action, spectral image analysis was applied to diagnose plant responses to herbicides and thus to diagnose HR by comparing spectral image responses of suspected HR plants with that of susceptible reference plant at various plant growth stages. Two spectral images, thermal and chlorophyll fluorescence images, were acquired at early stages after herbicide treatment and analyzed using MATLAB software to assess plant body temperature and photosystem (PS) II quantum yield, respectively. Plant body temperature increased with herbicide dose, while PSII quantum yield decreased. The comparisons of changes in plant body temperature and PSII quantum yield measured at 2-4 days after herbicide treatment with plant biomass change measured at 3 to 4 weeks after herbicide treatment revealed that spectral image analysis could detect herbicide response and discriminate between R and S plants right after herbicide treatment, much earlier than conventional whole plant bioassay. Our results thus suggest that spectral image analysis can detect HR much earlier at various growth stages than existing diagnostic methods, so farmers can effectively arrange alternative solutions for HR weed management.

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## **Discovery and development of novel herbicides by combining biological and chemical rationales with computational-technologies**

Avishag Levi Bar-Shalom  
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We are aiming at the improvement of crop productivity for the food, feed and fuel industries. For this purpose, a proprietary technology platform was developed; leveraging scientific understanding and computational technologies for introducing improved seed traits, innovative ag-chemicals and novel ag-biological products. Innovative and robust computational platforms (PoinTar™ ; PointHit™) for the discovery and assessment of novel essential protein targets and potential specific small molecule modulators for these targets. These platforms have already been utilized for novel plant protein target discovery and design of small molecules libraries that resulted in bio-validated novel herbicide hits and new herbicide modes-of-actions. Such discovery capability provides the foundation for generation of an internal novel herbicide pipeline. Ten chemical groups have now demonstrated initial positive results in our screening assays in the lab and greenhouse. These compounds were computationally predicted to impact six essential targets for their herbicidal activity in novel mode-of-actions. Another key activity, includes the establishment of a computational infrastructure addressing key parameters for molecule optimization such as efficacy, toxicity, and resistance evolution in weeds. In this respect, we are perusing different optimization candidates such as improve efficacy/toxicology issues of a non-selective major herbicide. At present, we are collaborating with leading partners throughout the world, leveraging our unique approach and tools to bring innovative and safe herbicides to the market.

## **Integration of herbicide band application and inter-row cultivation in maize using RTK-GPS systems**

Donato Loddo, Laura Scarabel, Maurizio Sattin  
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Reducing herbicide use is an important step to decrease environmental impact and the risk of herbicide resistance evolution by reducing selection pressure on weeds. Herbicide application localized along the crop row can contribute to lower chemical input for weed control. A field experiment was conducted at CAB Massari farm (Conselice, RA, Northern Italy) to evaluate herbicide band application systems for silage maize.

Treatments consisted of post-emergence herbicide band application (T1, mesotrione and prosulfuron at 30 and 7.5 g ai/ha corresponding to 50% of label dose, prototype inter-row cultivator, nozzles Tecsi 02-110, pressure 2 bar, volume of spray 180 L/ha), pre-emergence band application (T2, thiencazzone-methyl and isoxaflutole at 12 and 30 g ai/ha corresponding to 33% of label dose, Gaspardo seeder, nozzles Teejet TP0802EVS, pressure 2 bar, volume of spray 100 L/ha) and pre-emergence broadcast application (T3, thiencazzone-methyl and isoxaflutole at 36 and 90 g ai/ha corresponding to full label dose, Barigelli sprayer, nozzles Teejet TP11002VP, pressure 3 bar, volume of spray 200 L/ha). Inter-row cultivation, fertilization and irrigation were applied similarly for all treatments. Weed samplings were conducted before and three weeks after post-emergence herbicide application and at crop harvest to evaluate weed density, botanical composition and control efficacy.

Weed density in untreated areas ranged between 15 and 30 plant/m<sup>2</sup> with the presence of typical summer species (*Solanum nigrum*, *Amaranthus* spp., *Chenopodium album*, *Convolvulus arvensis*). Optimal weed control and good yields (about 7.5 and 2.5 ton/ha of fresh and dry matter) were achieved without significant differences for all tested systems, underlining the feasibility of herbicide band application integrated with inter-row cultivation for low chemical input weed control in silage maize.

This study was funded by the Emilia Romagna region within the Rural Development Plan 2014-2020 Op. 16.1.01 – GO PEI-Agri - FA 4B, Pr. »Resistenze« and coordinated by CRPV.

## **Assessment of black-grass (*Alopecurus myosuroides* Huds.) densities using airborne imagery**

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The steadily increasing number of resistant black-grass (*Alopecurus myosuroides* Huds.) populations and the adverse impacts of climate change, exacerbate plant protection year by year. To monitor these effects, the University of Hohenheim has conducted collaborative field experiments with the State Plant Protection Services since 1969. The efficacy of crop specific herbicide mixtures have been tested in maize, soybean, winter barley and winter wheat against present weed populations. The current study was conducted in winter barley fields at Wurmberg with a high infestation of about 900 *Alopecurus myosuroides* plants m<sup>-2</sup>.

The aim of this study was to correlate vegetation indices, from airborne imagery with ground truth field data, like crop and weed density after the herbicide application in autumn when *A. myosuroides* plants had 4 to 7 leaves and in spring at tillering. The Unmanned Aerial Systems (UAS) were used to take georeferenced high-resolution pictures with a spatial resolution of 3 cm of a winter barley field in southern Germany (Wurmberg), during the 2016 / 2017 cultivation season. The sensors used was a typical RGB camera. The Excessive Green (ExG), and the Excessive Green Red (ExGR) indices were calculated, based on the UAS RGB images. These indices were compared with the measured ground truth data of weed and crop density. Ground truth was gathered by grid sampling inside the field at four randomly chosen places per plot. The ExGR index value correlated with the *A. myosuroides* plant density at two flight dates both at mid of November and at the end of March (R<sup>2</sup> of 0.4 and 0.49 respectively). Even though using threshold-based vegetation indices like ExGR cannot provide information about the actual weed species, they can show indications of weed infestation which can be valuable for weed predictions.

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### **Research needs for future weed management in Australian agriculture**

Arslan Peerzada, Chris O'Donnell, Steve Adkins  
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Large-scale adaptation of conservation tillage, increasing area under herbicide-tolerant crops, over reliance on herbicides, lack of diversity in herbicide-based weed management in combination with prevailing climate change scenario intensifying the weed problems in Australia. Increased dependence on herbicides resulted in the frequent and widespread evolution of herbicide-resistant weeds as well as weed population shifts in Australian cropping systems. Ensuring diversity in weed management through the integration of improved weed management techniques (e.g., harvest weed seed control, weed seed catchment, burning) will reduce the selection pressure on weeds, which minimizes the evolution of herbicide resistance and will prevent their shifts towards dominance or difficult-to-control weeds. Increasing crop competitiveness through altering crop management practices need to be explored more broadly as a potential option for sustainable weed management. Development of precision weed management technologies for herbicide spraying and targeted tillage might help in reducing herbicide usage and increase profitability. In addition, formation of a fully integrated weed-activated mapping and spraying system is in progress and is advancing rapidly. Significance of allelopathy for weed management in agricultural systems, either in the form of allelopathic cultivars or plant-derived chemicals, cannot be neglected; however, the use of allelopathy is still in an infant stage in Australian agriculture. In addition, weed seed predation through insect pests and pathogens will open up diverse ways for the biological weed management and the development of bioherbicides or mycoherbicides. Furthermore, a nationally coordinated approach involving all government levels in collaboration with industries, landholders, and the community is required to establish appropriate legislative, educational, and coordinated frameworks against weeds.

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## **Yield and quality of *Abutilon theophrasti* as fiber crop for use of renewable resources**

Jan Petersen

University of Applied Sciences Bingen, BINGEN, Germany

*Abutilon theophrasti* is known as an important weed in many regions. However, it is a weed in many parts of the world because it was grown as a fiber crops in former times. In recent times renewable resources are of increasing interest. Plant species which are easy to grow and furthermore show good quality and yield are potential candidates for non-food use. But knowledge how to grow *Abutilon* is not present anymore in Europe.

In Southwestern Germany field trials with *Abutilon theophrasti* were conducted in three years (2015-2017). Aim was to investigate the yield potential and the fiber quality depending on different parameters like plant density, fertilization, row spacing and water irrigation.

Main result was that *Abutilon theophrasti* can be grown in SW-Germany as a fiber plant. At plant density of 30 plants/m<sup>2</sup> fiber yield was up to 1.5 t/ha. Fiber quality was comparable to other plants like hemp. *Abutilon* is tolerant to drought. However, for a valuable yield a sufficient water supply (April to August > 250 mm) is essential. Crucial is the seed rain of *Abutilon* which can result in a significant impact into persistent weed seed bank. Furthermore, a valuable use of plant residues after fiber extraction must be found before *Abutilon theophrasti* can be grown as a crop in Europe.

### **Weed identification by mobile device**

Hans-Joachim SanteJ, Sebastian Hellweg, Daniel Rechsteiner, Marek Schikora  
Bayer AG, LANGENFELD, Germany

An array of mobile device-based applications for field status monitoring, problem identification, and decision making on crop management is under development at Bayer AG, including an identification system for most important weeds at young development stages typically found in major annual field crops. The user takes an image of the weed in question with a smart phone in the field. The application excises the core plant area and uploads this data to a host computer for further processing. There, image analysis is performed using deep residual neural networks. For a recognition of the characteristics of the weed, a multitude of evident morphological plant features including leaf shape, color scheme, surface structure and texture, edge to area ratio of leaves, but also less tangible features are examined. The algorithm compares such information with features learned from images used for its training on the range of possible appearances of individual plants of a given species. For the weed identification algorithm thousands of proprietary images from natural and controlled environments have served as a training resource of the possible appearances of weeds covering development stages from cotyledons to advanced stages. The accuracy of identification is influenced by several factors including size, quality and variety of the set of training images as well as the singularity of plant features depicted in the image of the weed in question. As an output the system proposes a number of species for which the algorithm has calculated high conformity with trained weed data. This result is transferred back to the user's device and can serve as input into pending decisions on crop management. Identification delivers very high precision of identification for weed species with a relatively unique appearance like *Galium aparine*, and becomes more challenging within a genus like *Matricaria* with many similar looking species

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## **Sensors-based detection of weed response to application of herbicides**

Tal Shilo

Newe Yaar Research Center, RAMAT YISHAY, Israel

The decreasing inventory of available herbicides in the market is a critical issue that can be mitigate by reducing failures in weed management and establishment of resistant populations. Being able to objectively estimate the success or failure of applied weed control tactic in the field can serve as a powerful tool in optimizing management protocols. The goal of the current research was to develop a remote-sensing based pipeline to evaluate weed response to herbicides application. To evaluate the viability of weeds after application of herbicides we have collected data using an unmanned aerial vehicle (UAV) carrying an RGB sensor, as well as a commercial multispectral sensor that measures reflectance in the near infrared (NIR) and red-edge (REG) bands. Combining both data sets, we were able to produce maps that accurately represent the dispersion of weeds in the field. In addition, an index was developed to scale the success of weed control after application of an herbicide. This pipeline can help growers overcome bias of visual evaluation, spot local problems in the efficacy of the control method that was implemented and therefore manage their control strategy more accurately and successfully.



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**RoboWeedMaPS - Automated species detection and variable, precise targeting**

Gayle Somerville, Bo Melander, Solvejg Mathiassen  
Aarhus University, SLAGELSE, Denmark

Herbicide is an essential weed control tactic in many farming systems, however herbicide use is becoming more regulated, and more expensive. In addition, the widespread and more frequent use of herbicides has been linked to the evolution of herbicide resistance. The aim of this project is to improve the use of Deep Learning (DL) and Big Data in weed control planning, and thereby reduce herbicide use, whilst improving knowledge of weeds, and their control. This project has started with the development of sensory and deep learning systems for weed analysis. RoBoWeedMaPS will also include sociological surveys of farmers' reactions to the new technology. Products will be developed that can be used both individually and in an integrated manner. The integrated system will achieve a single coherent chain of actions, resulting in significant synergies and »fit«. Vis: weed detection storage in new farm management information systems (FMIS) optimised selection of herbicides site-specific applications of herbicide mixes and doses documentation of herbicide products and amounts to the FMIS. The use of these systems will reduce herbicide consumption by 20-80%, and produce maps of weed locations, size, and herbicide susceptibility. Further, the project will develop new camera techniques, spray equipment and software for weed recognition and mapping.

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## **In-season discrimination of herbicide resistant and susceptible weeds by means of vegetation indices**

Ilias Travlos, Spyros Fountas, Dimitrios Bilalis, Nikolina Cheimona, Evangelos Anastasiou, Anastasia Tsekoura

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Precision agriculture and particularly sensor technologies have been already used for weed detection and measurement of weed infestation level. Moreover, quick tests are crucial for the rapid detection of herbicide resistant weeds and consequently their effective and timely management without the risk of a further dispersal. The objective of the present study is to discriminate *in situ* the resistant from the susceptible weed populations by means of canopy sensor derived vegetation indices (NDVI and NDRE). For that reason 20 populations of rigid ryegrass (*Lolium rigidum*) were sown and sprayed at 3-4 leaves with several herbicides at maximum recommended rates. Based on NDVI and NDRE data and taking into account the corresponding values for two standard populations (a confirmed resistant and a susceptible one), populations were characterized very early as resistant or susceptible. This classification was compared with the findings of a parallel pot experiment and the pros and cons of such an approach are discussed in the present study, giving in many cases the time and the opportunity of a quick reaction and an adequate weed control during the same growing season.

### **Weed modeling using close range photogrammetric imaging techniques**

Dionisio Andújar, Mikel Calle, Ángela Ribeiro, José Manuel Pena, Cesar Fernández-Quintanilla, José Dorado  
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Plant phenotyping allows researchers to gather plant architecture which is fundamental to improve plant characterization, selection or discrimination. Three-dimensional (3D) modeling using new sensors and software allows assessing and characterizing morphological parameters of plants. Structure from motion (SfM) is a photogrammetric range imaging technique for estimating 3D structures that reduces cost with high resolutions models when comparing with other sensors. This paper describes a procedure to characterize *Xanthium strumarium*, *Datura ferox* and *Sorghum halepense* plants using SfM. From two-dimensional images and computer vision a point cloud and a solid model was created. The model could be used for plant parameter extraction such as stem length, leaf area index (LAI) or leaves angle. A digital camera (Nikon D5500) was used for image acquisition. A sequence of images from top to ground was utilized for plant reconstruction. The correspondence between images and the reconstruction of 3D was guaranteed by a high overlapping between images. A total of ten individuals per weed species were selected in a maize field at the experimental farm »La Poveda« (Arganda del Rey, Madrid, Central Spain). The values of dry biomass and LAI were calculated after taking images. Every plant was properly reconstructed with only small parts missing. Results showed a good relationship between the modeling predicted values and the values observed in the field. The number of leaves was properly estimated in most cases without significant differences between models and observed values for each species weed. Indeed, *X. strumarium* and *D. ferox* showed correlation values higher than  $R^2=0.98$ , while *S. halepense* correlation values were slightly lower. These results suggest that weed plant reconstruction in great detail is possible with a simple and low budget system, which could be used in several scenarios, even for phenotyping processes.

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### **Sensor guided mechanical intra-row weed control in sugar beets**

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Due to environmental concerns and legal constraints regarding herbicide usage, different approaches to mechanical weed control in sugar beets have reemerged. The main problem of mechanical weed control is its inability to perform intra-row weed control. On that front finger weeders were introduced as a tool capable of intra-row weeding. Conventional finger weeders (CFW) are propelled by the forward movement of the tractor. They remove intra-row weeds. Their action is non-selective and crop damage may occur. Our aim was to examine if we could improve the finger weeder efficacy compared to mechanical and chemical weeding. In this field experiment, a modified finger weeder (MFW) for intra-row weed control was combined with an imaging sensor for sugar beet plant identification. The MFW were equipped with an electric motor. This enabled rotational movement of the finger weeders, independent of the driving speed. In combination with the imaging-sensor, different rotational speeds were realized. The intra-row space between two sugar beet plants was treated either at a higher (2.5km/h) or lower (1.8km/h) rotational speed than the crop plant itself. Tractor driving speed was 2km/h for all treatments. The treatments, included three times hoeing with a combination of CFW or MFW, two herbicide applications and the combination of an herbicide application and two times mechanical weeding. The experiment was performed during 2017 in south Germany. High weed counts were registered in all plots, with *Chenopodium album* and *Matricaria chamomilla* being the most abundant weed species. Average yield results with MFW were 48t/ha indicating a higher, yet non-significant, sugar beet yield than CFW (36.2t/ha). Combining MFW with a single herbicide application resulted in the highest yield with 77.9t/ha. Crop plant identification can help in decreasing crop damage and thus increase yield. Further development of the system can be promising for herbicide savings while maintaining high yields.

**S05-O Invasive Species  
ORAL PRESENTATIONS**

## **Stocking and controlling the invasive *Heracleum sosnowskyi* Manden. in southern Poland**

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*Heracleum sosnowskyi* is one of the most dangerous invasive species in Europe; it was introduced to Middle and Eastern Europe from Caucasus, during the 1950s, as a pasture plant for cattle. Presently it grows intensively, creating a highly dense stands, where no other plant species can grow. This species, due to a presence of furanocoumarins poses a significant threat to humans and animals health. The project entitled PL02 »Environment clear from *Heracleum sosnowskyi* (Manden.)« aimed at stocking and controlling *H. sosnowskyi* in southern Poland. This project is co-financed from EEA grants and is coordinated by the Faculty of Agriculture and Economics, University of Agriculture in Krakow. Stocking of *H. sosnowskyi* populations in southern Poland is carried out since 2014. The presence of *H. sosnowskyi* was stated in 31 municipalities of southern Poland on the area of 134 ha. This species was mostly observed along riverbanks e.g. Dunajec, Raba, Biała Tarnowska. As the project's output, this species was destroyed in ca. 80% of the stocked area.

In the years 2005-2014 field experiments were carried out in southern Poland, to compare the effectiveness of different methods of *H. sosnowskyi* control. The herbicides (glyphosate and flazasulfuron) or plant or root cutting were applied. All the methods were applied at different intensities, e.g. cutting/spraying once, twice or three times per season.

As a result, it was found that a long-term shoot cutting is ineffective for the control of *H. sosnowskyi*. Total control of this weed is obtained either by cutting the roots of plants up to 5 years old at a depth of 15 cm or by continuous (5 years long) herbicide spraying three times during the vegetative season, using a tank mixture of glyphosate and flazasulfuron.

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## **Biological Responses of Parthenium Weed to Different Environmental Factors Are Associated with Its Invasiveness**

Ali Bajwa, Bhagirath Chauhan, Steve Adkins  
University of Queensland, GATTON, Australia

Parthenium weed (*Parthenium hysterophorus* L.) is one of the most invasive weed species around the world. It poses serious threat to crop and livestock production, native plant biodiversity, human and animal health, and ecosystem stability. However, the invasion mechanism of this troublesome invader is not well known which makes its management further difficult. A series of experiments was conducted under controlled conditions to understand the biological responses of parthenium weed to different environmental factors. The morphological, physiological, and biochemical responses of this species were found to be strongly associated with its invasiveness. An invasive biotype of this weed was more efficient to respond the environmental fluctuations as compared to its non-invasive counterpart. This invasive biotype of parthenium weed was also superior in terms of the germination ability, growth, physiological regulations, biomass accumulation, and the reproductive capacity under different levels of the selected environmental factors including temperature, soil moisture, light, and atmospheric carbon dioxide (CO<sub>2</sub>). In general, parthenium weed plants adapted to sub-optimal conditions by maintaining their growth and reproductive capacity through physiological regulations. Plants tolerated the stress conditions by producing high levels of protective biochemicals including soluble proteins, soluble sugars, phenolics, and proline. Moreover, parthenium weed plants almost doubled their growth and biomass at elevated CO<sub>2</sub> (700 ppm) both under normal and drought environments. Several primary and secondary metabolites were also up-regulated under elevated CO<sub>2</sub> which helped plants to grow vigorously and accumulate more biomass. In conclusion, the ability of parthenium weed to grow and reproduce under a wide range of environmental conditions enables it to invade and establish in diverse areas.

## Aquatic invasion corridors of *Conyza canadensis*, *Echinochloa crus-galli* and *Xanthium strumarium* in Serbia

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*Conyza canadensis* (L.) Crong, *Echinochloa crus-galli* (L.) P. Beauv and *Xanthium strumarium* L. are among the most important weed species in ruderal and arable areas of Serbia, categorized as highly (*C. canadensis* and *E. crus-galli*) and potentially invasive (*X. strumarium*). Since watercourses act as important transportation routes in the long-distance dispersal of weeds, our aim was to analyze the degree of invasion of these three invasive weeds in riparian areas and examine the role of rivers and canals as their potential invasion corridors.

Field research was performed over the summer months of four years (2013-2016), along the course of 37 rivers and seven major canals of the Danube-Tisa-Danube hydrosystem in Serbia. Fieldwork was performed in 230 field sites, along 100 m long transects, where the presence and abundance of invasive alien weed species were recorded.

Over the period of four years, the presence of *X. strumarium* was registered along the course of 35 rivers (95%), with a maximum cover reaching 100% of the river transect. *E. crus-galli* and *C. canadensis* were recorded along the course of 28 rivers (46%), with a maximum cover of 80% and 70%, respectively. Of the total number of field sites (230), the presence of *X. strumarium* was documented along 65%, while *E. crus-galli* and *C. canadensis* were recorded along 46% and 42% of field sites, respectively.

The data on the distribution of these alien invasive weeds expands the existing knowledge on their presence in arable and ruderal areas of Serbia, while at the same time highlighting river riparian areas as potentially important corridors of their further spread.

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**Strategies to eradicate *Ailanthus altissima* (Mill.) Swingle in a forested area**

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*Ailanthus altissima* is a tree belonging to the Simaroubaceae family. Its high invasiveness potential may have a strong impact on the biodiversity in non-native areas. The aim of this study was to test practical and effective chemical methods to eradicate spot infestations of *A. altissima* in a forested area. The study, started in summer 2016, was conducted in a forest ecosystem located in the municipality of Almese, North-west Italy. Two representative infestation spots (A and B) have been individuated and *A. altissima* plants counted and labeled. A total of 76 (A) and 82 plants (B) were considered. The herbicides used were glyphosate (as 540 g/L formulated product, diluted at 50% v/v) and a mixture of aminopyralid+fluroxipyr (144.1 g/L+35.5 g/L; diluted at 10% v/v). Two application techniques were compared: basal bark application (BB) and stem injection (SI). BB technique was applied on plants with a circumference lower than 12 cm moistening the first 0.5 m of plant stem by using a hand pressure sprayer. SI technique has been applied on plants with circumference higher than 12 cm. Herbicide was injected into plant stem through holes previously made with a battery drill. The number of holes varied in accordance to the circumference size (2 holes for circ. 12 to 20 cm, 4 holes for circ. 21 to 40 cm). One year after treatment, about 70% of plants treated with aminopyralid+fluroxipyr showed living sprouts, with negligible differences among the application technique used. Only 30% of plants treated with glyphosate showed living sprouts, the majority of them treated with BB technique.

### **What makes *Ambrosia confertiflora* an efficient invasive weed?**

Yifat Yair, Moshe Sibony, [Baruch Rubin](#)

The Hebrew University of Jerusalem, REHOVOT, Israel

Plants of the genus *Ambrosia*, specifically *Ambrosia artemisiifolia*, are known throughout the world as invasive, allergenic, and noxious weed. Five *Ambrosia* species were identified in Israel in various phases of invasion. The invasive *Ambrosia confertiflora* DC; three naturalized species: *A. tenuifolia* Spreng, *A. psilostachya* DC and *A. grayi* (A. Nelson) Shinnars, and the casual species *A. artemisiifolia* L.. *Ambrosia confertiflora* originating from southern US and Mexico, is a very aggressive and competitive perennial weed. It was first recorded in Israel at 1990 and underwent a population explosion in less than 15 years. *A. confertiflora* employs several strategies that make it an efficient invasive weed. Many viable achenes, absence of seed dormancy or stratification and dispersal by animals (zoochory). Plants grow rapidly from rhizomes and occupy large areas. This plant exhibits flexibility in its growth under different temperature conditions and this allows for effective competition with nearby plants. Plant development was studied under four different temperature regimes: 28/34°C, 22/28°C 16/22°C and 10/16°C n/d. Plants and achenes were grown in each regime and shoot elongation, biomass production, numbers of sprouts and seed germination were measured every week. Results indicate that under low temperatures (10/16°C and 16/22°C) shoot elongation is inhibited, plants form rosette and sprouts number increase. Biomass production varies at different temperature regimes, and is higher under warm conditions (22/28°C and 28/34°C) and the plants elongate quickly, achieving an advantage in the competition for sunlight and better opportunity to disperse pollen and seeds. These characteristics allow *A. confertiflora* to thrive in different environments and provide an advantage over other *Ambrosia* species.

## **S05-P Invasive Species POSTER PRESENTATIONS**

## Results of 3-years survey on spreading of *Ambrosia artemisiifolia* L. in Ostrovné lúky, Slovakia

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Results of 3-years survey on spreading of *Ambrosia artemisiifolia* L. in Ostrovné lúky, Slovakia

The most important weed species within Europe – especially in the Pannonian Plain in Central Europe – is the invasive common ragweed (*Ambrosia artemisiifolia* L.). The object of this study is the field survey taken during summer 2014, 2015 and 2016 in Ostrovné lúky (Žitný ostrov, Slovakia). This region belongs to Natura 2000. Natura 2000 is a network of protected sites of the European Union member states. The main reason for establishment of the network is an effort to maintain European natural heritage. Invasive species have a negative impact on the natural ecosystems. Monitoring of invasive plant species is a very important task. On the prepared map, we placed a grid net. The randomly selected areas based on plots of a standard size of 10 m<sup>2</sup>. An 11-point scale was used for the assessment of population density and the subjective category of presence varied from weak to heavy infestation on this arable land (mostly stubble fields). To understand the distribution of ragweed in the territory a geospatial analysis was applied to create 2D map in ArcGIS environment. The geospatial analysis enables not only to clearly understand frequency and dominance of ragweed in the territories, but it also offers useful data to forecast potential *Ambrosia* spreading. However, the number of weeds on plots was very different and the deviation in the several years was different, the statistical analysis has shown that the average of the infected ragweed samples from this land belonging to Natura 2000 in the years of 2014-15-16 were not significantly different ( $p > 0.05$ ).

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## Temperature-based prediction model for *Ambrosia confertiflora* development

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*Ambrosia confertiflora* is an invasive perennial weed considered among the most troublesome weeds worldwide. This noxious weed causes severe damages in agriculture, nature reserves, roadsides and disturbed areas. The species has two reproduction organs, the seed and a vegetative rhizome organ. Since the biological data about this weed is limited, our objective was to develop thermal growing-degree-day (GDD) models for predicting seed germination and rhizome emergence under constant temperature regimes. For germination, the base temperature was 6°C, with an optimum temperature of 19°C. Seed germination was completely inhibited at temperatures over 36°C. Germination under controlled conditions was best explained as a function of GDD by a sigmoidal Log-logistic regression (RMSE=5.73). According to the prediction model, germination began at 50 GDDs and maximal germination occurs at 540 GDDs. For rhizome emergence, the base temperature was 11°C, with an optimum temperature of 17°C and maximum temperature of 28°C. This process was best explained as a function of GDD by a logistic equation (RMSE=10.54). According to the prediction model emergence from rhizome began at 30 GDDs and continued until maximal emergence at 120 GDD. Rhizomes were also buried in different depths: 0, 2, 4, 6 and 8 cm to calculate the thermal time for the production of new shoots from different depths. Shoot emergence from rhizomes was best explained as a GDD function by a three-parameter logistic equation (RMSE=3.22). According to the prediction model, 110 GDDs after planting, new shoots began to emerge from all examined depths (plowing layer). From these prediction models, it is clear that the temperature is in strong association with the above ground and sub-surface development of *A. confertiflora*. The quantification of the different interactions on a time scale of GDD can serve as an efficient tool for *A. confertiflora* control.

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## **Biology, impact, and management of serious invasive weed, *Sonchus oleraceus* L. in Australia**

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The status of *Sonchus oleraceus* was forced to be raised from relative obscurity to a most widespread, troublesome and economically damaging invasive weed in Australia, particularly in the northern region from central Queensland to northern Wales because of important shifts towards conservation tillage systems. Over the last 15 years, evolutionary herbicidal resistance, genetic diversity, prolific seed production, low level seed dormancy, and absence of natural predators contributes towards *S. oleraceus* success as 5<sup>th</sup> most difficult-to-control invader weed. Its seeds are capable to germinate under varying temperature and light condition and possesses the ability to germinate throughout the year in northern regions. Furthermore, seedling emergence, favoured by moist environment, is observed greater in seed present on or near the soil surface in no-till systems. Several populations have been discovered resistant to numerous herbicides, including chlorsulfuron, atrazine, and glyphosate. However, carfentrazone, florasulam, flumetsulam, bromoxynil octanate, tribenuron methyl, bromoxynil, and sulfentrazone singly or in mixture forms have been found effective in suppressing the growth and reproduction of *S. oleraceus* in small infestations. In addition, double-knock tactic would be considered an effective approach to achieve high level weed control through preventing seed set. However, integration of weed control approaches has been reported to be more reliable and efficient for the long-term control of *S. oleraceus*. This article was intended to highlight the current scenario and future prospects of this economically damaging invasive weed, particularly in Australia. Understanding related to the physiological aspects regulating the invasion biology of *S. oleraceus* will help in predicting its agro-ecological impacts and has pragmatic implications in designing management strategies.

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## **The biology, distribution and management of the invasive weed *Parthenium hysterophorus***

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*Parthenium* weed (*Parthenium hysterophorus*) (Asteraceae; Heliantheae) is an invasive allergenic species, that invaded many countries worldwide, probably with imported livestock feed. In Israel, it was first reported in Bet-She'an valley and rapidly moves to the Western Jezreel valley. *Parthenium* is an annual weed that grows and flowers throughout the year, infesting field and vegetable crops and orchards. Contact with the weed may cause serious allergenic reactions to humans and animals as well as damaging the ecosystem by threatening the biodiversity, and serving as a host to pests and pathogens. In addition, as *parthenium* is a quarantine plant, any contaminated produce exported will be rejected by the European market. The aims of the current study were to document the current distribution of the weed in Israel and map its distribution over time. We also aimed to study its life cycle and optimize its management using chemical and non-chemical control methods. In this research, we have detected rapid invasion to new areas and habitats in the Jezreel valley, the Jordan valley and the cost line of the Mediterranean Sea. Studying the biology of the weed, we have found that the optimal temperature regime for seed germination was 28/22 °c (day/night). Moreover, *parthenium* seeds were able to emerge only from a depth of 0-3 cm. *Parthenium* was found very sensitive to the herbicides diuron, atrazine, metribuzin, clomazone and glufosinate, applied pre- and post-emergence. We plan to integrate this accumulated knowledge regarding the biology, phenology and response to herbicide to establish a sustainable management program that could reduce its detrimental impact on crops and the environment.

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## **Different control measures against *Ambrosia artemisiifolia* on a natural infested roadside in East Germany**

Ulrike Sölter, Arnd Dr. Verschwele, Uwe Dr. Starfinger  
Julius Kühn-Institut, BRAUNSCHWEIG, Germany

A large scale field experiment with four replicates on a roadside banquette in Brandenburg (federal state of East Germany) with a natural common ragweed (*Ambrosia artemisiifolia*) infestation was carried out. Thermal control treatments were hot water (Wave High Series hand unit, Wave Europe, Wekerom, Netherlands) and flaming (Green-Flame 850 E, Green-Flame, Vordingborg, Denmark), the mechanical treatment was mowing (selfpropellered mower by road maintenance staff) and the chemical treatment was the application of Banvel M (6 L/ha; 30 g/L Dicamba, 340 g/L MCPA). The experiment was conducted at BBCH 50-65 of *A. artemisiifolia* in 2011, 2012 and 2013. *A. artemisiifolia* plants were counted 4 weeks after the treatments, in 2011 the assessment failed due to technical problems. The impact of the treatments as a long-term effect on the soil seed bank was investigated by taking soil samples in June 2014 and conducting a germination test in summer 2014 and in spring of each year in 2015, 2016 and 2017. Due to a very high variance of the results no significant differences were detected. But regarding the results of the soil seed bank germination test, obviously a reduction of *A. artemisiifolia* seeds was achieved by the treatments hot water, herbicide and mowing in comparison to flaming and the untreated control.



### **Management of invasive neophytes on cropland to ensure its productivity**

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The spread of invasive neophytes is increasingly threatening the productivity of agricultural areas. The aim of the project ENVISAGE (time span 2016-2019) is to provide improved fundamentals for effective control of 9 invasive neophytes that are agriculturally relevant. Exemplarily results on trials with Russian Olive (*Elaeagnus angustifolia*) from the first experimental year are discussed. 4 different mechanical control measures were conducted on an extensive grassland with shrubs and trees where Russian Olive is growing. The treatments were: 1) 5 times mowing, 2) 5 times mowing and subsequent harrowing, 3) 2 times digging over, 4) 5 times rolling over. Treatment 1 and 2 gave the best results concerning low coverage at the end of the vegetation period. Beside these on-site experiments, small scale herbicide trials with shoots taken from Russian Olive were also conducted. Two herbicides were tested on 5 plants per herbicide after cutting them down to an equal height of 30 cm: 2 L/ha Garlon (150 g/L Triclopyr and 150 g/L Fluroxypyr) and 2 L/ha Simplex (100 g/L Fluroxypyr and 30 g/L Aminopyralid). Two months after application all plants were killed. Another small scale field trial was established in order to find out the potential risk of burying and also spreading above ground small parts (7 cm) of roots and young and old branches of Russian Olive. At the end of the vegetation period none of these small parts were budding. These first results show that there are promising measures to fight against Russian Olive.

**S06-O ALS-Tolerant Crops  
ORAL PRESENTATIONS**

## Do We Need More ALS-Tolerant Crops?

Baruch Rubin

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ALS inhibiting herbicides belong to five different chemical groups that share the same mode of action, namely inhibition of the enzyme acetolactate-synthase (ALS) or acetohydroxy acid synthase (AHAS). Almost 30 different ALS-inhibiting herbicides registered for use in Europe, applied on almost 115 million hectares annually throughout Europe and Former USSR. At present, ALS-inhibiting herbicides are an important component in almost all crop rotations, applied sometimes on most crop/year of the rotation. As expected, the selection pressure imposed during the 30 years of intensive use on the weed populations resulted in immense resistance evolution in both grass and broadleaved weeds. Resistance to ALS-inhibiting herbicides from all chemical groups is reported in 159 weed species around the world, of which at least 30 weed species were found in European countries. Important weeds such as *Alopecurus myosuroides*, *Apera spica-venti*, *Stellaria media*, and *Papaver rhoeas* evolved ALS-resistance infesting fields in more than eight European countries. The non-transgenic imidazolinone-tolerant crops («IMI-crops» or «Clearfield®») such as sunflower, wheat, rice, oilseed rape, corn and lentils are already grown on more than 10 million hectares throughout the world. In addition, some of the crops contain additional trait that endows also tolerance to sulfonylurea herbicides. A novel trait was recently published, describing a non-transgenic ALS-tolerant sugar beet, a major crop occupying almost 5 million hectares in Europe. These traits provide unique and improved weed management tools enabling efficacious control of specific and problematic weeds, but at the same time will inevitably increase the amount of these herbicides applied and the associated selection pressure on the weed populations. Hence resulting in threatening the future use of much valuable and important ALS-inhibiting herbicides. Special stewardship and consideration are required to protect the already fragile and precarious environment before irreparable harm occurs.

## **Resistant-weed management in Clearfield® crops - a European stewardship program**

Matthias Pfenning

BASF SE, Limburgerhof, Germany

Herbicide tolerant (HT) crops offer growers a greater number of options for weed control. In Europe, non-GM HT is an established weed management practice. Broad spectrum ALS-herbicides in tolerant crops have been rapidly adopted by growers, replacing or complementing soil residual herbicides in crops like sunflower and oilseed rape. With the increasing cultivation of HT varieties, the possible accelerated evolution of herbicide-resistant weeds has become a concern. Simplification of crop rotations and the repeated use of the same chemistry in a given crop rotation could further enhance the potential for selecting herbicide resistant weeds, unless careful stewardship programs are followed. Stewardship guidelines exist for both, the seed and chemistry ensuring high seed quality, as well as adequate crop tolerance with the recommended herbicides and dose rates. Crop specific stewardship programs proposed by BASF are an integral part of communication towards the Clearfield users to ensure the sustainability and the effectiveness of this technology for the long term. In Clearfield® sunflower the stewardship recommendation consists out of multiple pillars - crop rotation and the accompanying alternation of non-ALS herbicides. Limiting the sole reliance on ALS herbicides to no more than 2 out of 4 years in the same field and the use of sequential or tank-mix partner herbicide with other modes of action complement the recommendation in Clearfield sunflower and in following crops. Avoidance of fields with strong wild sunflower populations to reduce the risk of trait outcrossing is another stewardship element. For Clearfield® winter oilseed rape, stewardship is concentrating first on volunteer control in the crop rotation. HT oil seed rape (OSR) volunteers are controlled using appropriate non-ALS chemistry in break crops or by mechanical means after harvest as part of good agricultural practice. As part of the chemical stewardship, all Clearfield OSR herbicides include at least two different modes of action. The use of multiple weed management practices, such as crop rotation, mechanical cultivation and delayed planting is recommended. Preferable and where applicable, HT crop rotations should consist out of winter and spring crops to lower the selection pressure on any specific weed species with a proposed interval of more than two years. Stewardship implies that whoever sells or uses herbicides for ALS-tolerant crops exercises the precautions for minimizing any undesirable effect of the herbicide use including selection of resistant weeds. An ever-increasing challenge in a generic environment.

## Experience with ALS tolerant sunflower, oil-seed rape and sugar beet in Central Europe

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Commercialization of herbicide tolerant (HT) crops started in 1992 by introduction of maize tolerant to imidazolinone herbicides. Very soon, genetically modified (GM) varieties complemented and later on substituted this technology in many major crops all over the World. Europe and most African countries are very restrictive in registration and use of GM crops leaving non-GM technology as a sole chance how to utilize the advantages of HT technology including crop selectivity, flexible weed control timing, and an alternative to manage hard-to-control weeds. The Central European region is suitable for growing almost all temperate zone crops. Diversity of soil and climatic conditions and many regulatory restrictions in use of residual herbicides brings also a wide spectrum of weed infestation that requires a diversity of weed control solutions. This technology is most widely adopted and dominant in sunflower in Central Europe, due to high crop selectivity and more efficient post-emergence weed control. Clearfield<sup>®</sup> system (imazamox tolerance) is preferred to ExpressSun<sup>®</sup> (tribenuron tolerance) because of higher crop selectivity and better annual grasses control; on the other hand, the ExpressSun<sup>®</sup> system has a quicker herbicide efficacy. So far, Clearfield<sup>®</sup> HT is not largely adopted by oil seed rape (OSR) growers in Central Europe (1-3%) in spite of the comparable yield and quality of the current varieties. The reasons are probably a combination of tradition, sufficient choice of conventional herbicides and lack of alternative herbicide to control ALS-tolerant volunteers in subsequent crops. The farmers prefer to use HT technology in fields with occurrence of Brassicaceae, Geraniaceae, and Boraginaceae weed species, in rain-fed areas, and the advantage of post-emergence control of volunteer cereals in one pass. The new technology - Conviso<sup>®</sup> Smart - ALS tolerant sugar beet (SB) is expected in 2019. Currently, it is very difficult to prepare a combination of herbicides suitable for split application, which would satisfactorily control weeds without damaging the sensitive crop. Besides, the available herbicides for sugar beet are applied in high use rates, are expensive and do not have a favourable environmental profile. Our experience from field trials with Conviso<sup>®</sup> Smart shows much higher crop selectivity compared to conventional herbicides, better targeted weed control, reduced number of treatments, and excellent performance on weed beets, *Abutilon theophrasti*, *Aethusa cynapium*, *Mercurialis annua* and many other difficult to control species. ALS-tolerant crops are one of the few alternative options available in Europe to diversify weed management systems in crops, which face regulatory difficulties in the availability of efficient, selective, and environmentally sound herbicides. ALS-resistant weeds are not widely spread in summer annual broadleaved species, such as sunflower and sugar beet, which are not dominant in crop rotations, hence we do not expect substantial increase in ALS-resistant weeds evolution. Another situation could be with some winter annual weeds occurring in OSR, but the ALS-tolerant varieties are not expected to have a major share on total OSR acreage.

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## **ConvisoSmart to Control Weeds in Sugar Beet. Integrated Weed Management and Product Stewardship**

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ConvisoSmart is an ALS-tolerant sugar beet technology consisting of two parts: a seed trait and an herbicide. Herbicide tolerant crop varieties are convenient technologies for weed management and they need appropriate stewardship in order to maximize herbicide efficacy, preserve the technology, guarantee farmer yields and avoid the selection of resistant weeds. The herbicide tolerance trait of the new sugar beet variety is not a GM trait but was obtained by selecting rare naturally occurring changes in the gene for acetolactate synthase (ALS). In ALS-tolerant sugar beets the ALS-inhibiting herbicide cannot bind to the ALS enzyme responsible for production of essential amino acids resulting in normal sugar beet growth while weeds are controlled by the herbicide. This is a new solution to control weeds in sugar beet offering for that crop increased diversity in weed control. Like in all cropping systems, an appropriate stewardship strategy based on pro-active herbicide resistance management has to be implemented to sustainably insure optimal weed control. The measures developed are based on IWM principles where weed control is diversified with chemical and non-chemical techniques depending on the main crop rotations locally used by the farmers. Stewardship includes also diagnostic support by scrutinizing complaints and potentially resistant weeds as well as to provide tailor-made management advice based on these investigations. Applying the above mentioned IWM measures will allow the sustainable use of ALS-tolerant sugar beet and provide a new weed management options to farmers.

**S07-O Cultural & Physical Weed  
Management  
ORAL PRESENTATIONS**

## Optimizing cover crop management for biomass production and potential of weed suppression

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Cover crops have been identified as an efficient tool in non-chemical weed management strategies. Cover crops suppress weeds mainly by competing with them for resources. High production of biomass limits the access of weeds to light and thus reduces their growth. However, cover crop biomass depends on seeding rate, date and termination timing. This study was conducted in seven location (five states) in the Northeastern United States and replicated 3 years. Hairy vetch (*Vicia villosa* Roth), an annual legume, which is one of the most commonly utilized species due to its cold hardiness, was planted at two dates in autumn and at rates of 6 to 50 kg ha<sup>-1</sup>. The cover crop was terminated in the next spring at the stages of early, intermediate vegetative, and 50% flowering. Cover crop biomass was mainly determined by the total growing degree days (GDD) between planting and termination and increased by 529 kg.ha<sup>-1</sup> every 100 GDD. A nonlinear model (asymptotic regression through the origin) was used to fit the biomass data as a function of the seeding rate. The model included two parameters: *asym* was the asymptote (i.e., the maximum biomass when seeding rate approaches infinity); *lrc* was the natural log of the rate constant, representing how quickly the asymptote was reached. Only 3 out of 80 data sets (4%) could not be fitted to the model. Contour plots provided a visual demonstration of the interactions between seeding rate and seeding dates on hairy vetch biomass by termination timing. Thus, the study identified how much seeding rate needed to be increased when seeding date was delayed to maintain a given biomass production.



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### **Weed Control in Pacific Northwest Tree Nurseries Using Soil Solarization**

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Research was conducted at three sites over two years to determine if soil solarization for weed control in tree seedling nurseries is feasible in the cooler climate of the Pacific Northwest of the USA. Seeds of four species, *Amaranthus retroflexus*, *Poa annua*, *Polygonum pensylvanicum*, and *Portulaca oleracea*, were buried for 6 weeks at 5 and 10 cm in either solarized or nonsolarized plots. At all sites, both depths, and both years, solarization was most effective on *Polygonum pensylvanicum* and least effective on *Portulaca oleracea*, and increased dormancy of *Amaranthus retroflexus*. In the first year, more *Poa annua* seeds survived at 10 cm in the solarized plots versus the nonsolarized plots. In the second year, *Poa annua* was controlled at both depths in the solarized plots. Weed seedling emergence was reduced at all three sites in the solarized beds compared to the nonsolarized beds. For example, at one site average seedling emergence was 0.84 m<sup>2</sup> in the solarized plots compared to 21.04 m<sup>2</sup> in the nonsolarized plots. Reduction in hand labor for weed control in the following spring ranged from 55 to 69%. Results of this research indicate that solarization for weed control in the Pacific Northwest is feasible.

## Weed reduction potential of cover crop mixtures

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Weeds are a major determinant of grain yield reduction worldwide. Cover crops (CC), as a component of integrated weed management, can provide several advantages, like increasing the soil organic matter content, reducing the soil erosion and stabilizing the N content. Furthermore, CC are an opportunity to reduce the herbicide input into cropping systems, especially in spring crops.

The aim of this study was to analyze the potential of i) CC mono-cropping and ii) CC mixtures on weed reduction. The main idea was to i) use and ii) combine CCs to suppress weeds using only physical (e.g. *Phacelia*) or with reported allelochemical mechanisms (e.g. *Raphanus sativus*).

The experiments were conducted at the Agricultural Station Ihinger Hof from August until December 2016 and 2017 (Renningen, Germany). After winter cereal harvesting, the experiment was set up as a randomized complete block design. The development stages and the biomass accumulation of the different CC treatments were determined every second week. Weed amounts and composition, along with relative coverage were measured twice, 7 and 12 weeks after sowing.

As expected, high biomass CCs are more competitive and have a higher weed suppression outcome. CCs like *Raphanus sativus* (2016) and *Avena strigosa* (2017), which combine chemical and physical suppression mechanisms showed the best weed reduction results, between the mono culture treatments. Moreover, mixtures with a high content of allelopathic CC species were able to reduce weeds more effectively compared to other treatments.

The gained dataset helped to evaluate the effect of CC mixtures in comparison to mono-cropping as an option for biological weed control. Weed suppression was correlated with CC biomass and soil coverage, but combining different CCs with dual suppression mechanisms achieved even better results.

**Pre-harvest seed dispersal as an indicator of susceptibility to harvest weed seed control**Samuel Kleemann, Gurjeet Gill

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Presence of widespread herbicide resistance and increasing occurrence of several difficult to control weed species has forced many Australian growers to implement non-chemical weed control tactics. Harvest weed seed control (HWSC) practices including seed catchers and weed seed destructor can be effective on weed species that do not shed seeds until crop harvest. However, there is limited information available on seed retention and pre-harvest dispersal (shedding) pattern of most Australian weeds. A field study was conducted in 2016 to investigate seed shedding behaviour of 4 weed species until the crop was harvest-ready ( $\leq 12\%$  grain moisture). Plots of varying density of *Bromus diandrus* (brome grass), *Hordeum glaucum* (barley grass), *Bifora testiculata* (bifora) and *Galium tricornutum* (bedstraw) were established in wheat (cv. Mace). The pattern of seed shedding was determined by regularly collecting seeds from the seed traps placed in each plot.

There was a hyperbolic relationship between weed density and seed production ( $R^2 = 0.93-0.98$ ) for these weed species, with seed production ranging up to 12000-22000 seeds  $m^{-2}$ . *H. glaucum* was particularly prone to early seed dispersal and only  $<6\%$  of seeds produced were retained in panicles at crop harvest. Weed seed retention was much higher for *Bifora testiculata* (50%), *B. diandrus* (75%) and *G. tricornutum* showed no seed dispersal prior to crop harvest (100% retained). Even though *B. diandrus* had high seed retention (75%) until harvest, many panicles (30-80%) had lodged below the crop harvest height (15 cm). The severity of lodging in *B. diandrus* increased with weed density, which could be related to weaker stems at its higher density and this could be an important escape mechanism from HWSC. Based on the level of seed dispersal in this study, *H. glaucum* and *G. tricornutum* were the least and most suitable weed species for harvest weed seed capture, respectively.

### **The critical period of weed interference in upland rice in the Mid-West of Madagascar**

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In lowland rice, weeds are generally well controlled by submergence, line transplanting and mechanical weeding. In upland rain-fed rice, weed control is highly challenging especially due to a peak workload at the beginning of the rainy season and weeding is done manually. This study aimed at determining the critical period of weed interference in a low-input upland rice cropping system, in order to optimize the timing of weeding. During the rainy season 2016-2017, a field experiment was conducted in Madagascar, Ivory station (19°33.29'S, 46° 24.913'E). Eight different weeding regimes were tested: a group of early weed interference (weedy until 20 DAS [days after sowing], 40 DAS and 60 DAS); a group of late weed interference (weedy from 20 DAS, 40 DAS and 60 DAS); two controls (weed-free and weedy). The experimental layout was a randomized complete block design with four replications. Data collected were weed cover at 20, 40 and 60 DAS, rice grain yield and weed biomass at harvest. The average rice yield in the weed-free control was 2.020 kg.ha<sup>-1</sup>, and in the weedy control it dropped down to 10 kg.ha<sup>-1</sup> indicating more than 99% of yield loss. Early weed interference until 20, 40, and 60 DAS caused respectively yield losses of 6%, 60% and 87%; and late weed interference from 20, 40 and 60 DAS caused respectively yield losses of 63%, 39% and 9%. It indicated that weed presence before 20 DAS and after 60 DAS induced minor yield losses, while in-between these dates rice should be kept free from weed competition to avoid substantial losses. This information should advise farmers to better plan the timing of weeding, avoiding to invest labour in weeding when only a small increase in yield can be expected. This experiment will be repeated in the coming rainy season to confirm these results.

## Early Weed Suppression By Some Cover Crop Plants

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Weeds begin competition with crop plants in the very early stages and therefore, weed control must be done in early-season. In weed control, it is believed that cover crops has ability to suppress weeds in the early season after emergence. In spring 2015, weed control potential of sixteen cover crop species belonging to four families (*Brassicaceae*, *Fabaceae*, *Poaceae* and *Rosaceae*), which expected to be able to adapt to the corn-growing area conditions were investigated. The experiment was laid out in randomized complete block design with four replications. The blocks were composed of square parcels measuring 2 x 2 meters. After the emergence of both weeds and cover crops, all parcels were randomly surveyed and the weed coverage in 1 m<sup>2</sup> was recorded weekly and expressed as coverage area percent. As a result, when the weed coverage area was 82.5% in the control plot, the Fenugreek (*Trigonella foenum-graecum* L.), Egyptian clover (*Trifolium alexandrinum* L.), Salad burnet (*Poterium sanguisorba* L.), Red Pea (*Lathyrus cicera* L.) and Ervil vetch (*Vicia ervilia* (L.) Willd.) were the most weed suppressive treatments at the end of the first six weeks by 10,5%, 11,25%, 12,5%, 12,5% and 15% weed coverage respectively. Additionally, a highest soil coverage was achieved in the Fenugreek (*Trigonella foenum-graecum* L.) 76,25%, Red pea (*Lathyrus cicera* L.) 76,25%, Ervil vetch (*Vicia ervilia* (L.) Willd.) 70,00%, Salad burnet (*Poterium sanguisorba* L.) 67,50%, Egyptian clover (*Trifolium alexandrinum* L.) 65,00% and Common vetch (*Vicia sativa* L.) 65,00% treatments respectively. With today's herbicide resistance, the use of cover crops as an environmentally friendly alternative option should be extended to avoid the problem of pesticide residues.

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## **The impact of Growing Onions Under Straw Mulch on the Control of Weeds**

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In 2015, we have conducted a field trial to study the effects of covering onions with straw mulch as a weed control measure on the onion development, yield, weed populations and pest - disease attack rate. Plots arranged in randomised layout were covered with a straw layer of 4 or 8 cm thickness at three different dates (2., 17. and 25. April). Results obtained in plots covered with straw were compared with plots, which were treated with herbicides. Yield obtained at plots treated with herbicides was higher than yields obtained at plots covered with straw. Straw caused lowering of soil temperature and delay in onion development at the beginning of growing season. The highest yield among plots covered by straw was recorded at plots covered at the latest date with the thickest cover of straw. Straw mulch provided good control effect on annual weeds, but was not efficient against perennial ones. The covering of the onions with straw did not significantly influence the pest and disease attack rates, only small differences in pest attack rate between early and late dates of straw application were observed, and small differences in disease attack rate were recorded between different dates and thicknesses of straw mulch.

**S07-P Cultural & Physical Weed  
Management  
POSTER PRESENTATIONS**

## **Relation weeds of maize and intercrops in conditions of the climatically drier area**

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Cover crops offer an interesting option for maintaining soil fertility and improving conditions for subsequently cultivated maize growths. Incorporation of cover crops affects the environment as well as the development of maize and its weed infestation. The overall aim contributes to a clarification of relations between weed infestation of maize and applied cover crops. The field trial was conducted at the experimental field station in Zabcice (South Moravia, the Czech Republic). The average annual precipitation is 483.3 mm and the average annual temperature reaches 10.07 °C. Two variants of cover crop use were applied in the experiment. The first one with usage of cover crop (*Phacelia tanacetifolia*) was sown after the harvest of cultivated pre-crop. The second variant without cover crop, where the cover crop wasn't sown after harvest and soil was cultivated according to the different variants of soil tillage. Three options of soil tillage were used for each of the variant (conventional, minimum and strip tillage). The evaluation of weed infestation was carried out using a counting method in 2014 and 2015. The obtained data was processed by a multivariate analysis of ecological data (CCA). Twenty-two various species of weeds were found within the monitoring period. The results of the CCA are significant at the level  $\alpha = 0,001$ , for all canonical axes. The use of cover crops encourages an occurrence of perennial weed species (e.g. *Cirsium arvense*, *Convolvulus arvensis*, *Elytrigia repens*) and increases a risk of weed infestation by cover crop in maize. Strip tillage is likely changing the soil properties. In particular, the pace of soil warming has changed. Slower process of soil warming may lead to weed infestation by unusual weed species particularly for maize and especially by overwintering species (Project no. IP IGA 32/2017).



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## **Crop cover selection to improve weed control in multi-species agrosystems in Reunion Island**

Mathias Christina, Pascal Marnotte, Renaud Benard, Yoann Marin, Julie Courtois, Mickaël Virama, André Chabanne  
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Cover crops are increasingly used for weed management in tropical regions as an alternative to herbicide. But selecting the most suitable species of cover crop to be associated with a main crop requires long-term trials. Here we present a two-years set of experiments to assess the ability of various cover crops to limit weed growth.

First, a collection experiment of 55 species and varieties was performed to assess the life cycle of cover crops in tropical climate in Reunion Island, in three different sites. This experiment allowed us to select different cover crop species whose behavior would be adapted to the different agrosystems in Reunion Island (sugarcane in rotation or intercropping, arboriculture,...). Secondly, 10 species were selected and grown in large plots to assess their ability to limit weed growth in monospecific plots as well as mixture of cover crops.

After two months of growth, the most productive cover crops showed the ability to limit weed growth to fewer than 30% of the plot (e.g. crotalaria, oat, millet...) while the less productive were unsuccessful to cope with weeds. On the contrary, all combinations of two cover crops tested in this experiment were able to limit weed growth to fewer than 30% of the plot area.

Our experiment highlights some key cover crops adapted to intercropping and rotation in multi-species agrosystems as an alternative herbicide.

### **Effects of cultural method on weed control in organic soybean**

Irena Deveikyte, Zydre Kadziuliene, Monika Toleikiene, Vytautas Seibutis

Institute of Agriculture, Lithuanian Research Centre of Agriculture and Forestry, KEDAINIAI DISTR., Lithuania

Soybean (*Glycine max* (L.) Merr.) is an important grain legume and oilseed crop in many countries across the world. Weed infestation significantly decreases the yield and quality of soybeans. Cultural practices such as row spacing, sowing time, high seeding density and crop varieties have an effect on weed dynamics. The aim of this study was to determine the effect of row spacing in combination with different sowing times on weeds. The field experiment was conducted in 2016-2017 at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. Soybean variety Merlin was grown at two row spacings - narrow (25 cm) and wider (50 cm). Soybean was sown at two times: 1 - early sowing (middle of May) and 2 - late sowing (two weeks after the first time). The number and dry mass of weeds were recorded at the soybean BBCH 71-78 growth stage. During the study period up to 18-28 weed species were registered. Prevailing weed species were *Chenopodium album* L., *Capsella bursa-pastoris* (L.) Medik., *Thlaspi arvense* L., *Stellaria media* L., *Veronica arvensis* L., *Viola arvensis* Murray, *Tripleurospermum perforatum* (Merat.) M.Lainz. Weed species were affected by sowing time. The early sowing reduced the number of *T. arvense*, *V. arvensis*, *C. bursa-pastoris*, *S. media*, *T. perforatum* compared to the late one. The number and dry mass of annual and total weeds statistically significantly increased when sowing time was delayed. The sowing time did not affect the number and dry mass of perennial weeds. The response of weeds to row spacing was observed for total number and dry mass of weeds, where the highest weed mass (21 and 12 %) was recorded in wider rows. These differences were not statistically significant. Row spacing had no influence on weed mass.

**Relay cropping of winter cereals into summer perennial: a method of cultural weed control**

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Relay cropping is seeding of one crop into another standing crop. The practice of starting a winter cereal into an established pasture may reduce soil erosion, increase total production and assist with controlling weeds in cereal crops. In Mediterranean low-rainfall environments, some subtropical perennial grasses have been successfully grown because of their tolerance to dry summer periods and cold winters. In this study, we have relayed cropped winter barley into perennial C4 species to: i) evaluate the effectiveness of this practice in controlling weeds; and ii) assess the development and productivity of crops.

A randomized block design with four replications was established in the experimental farm »La Poveda« (Madrid, Spain). Winter barley was relayed cropped into four summer perennial grasses: *Brachiaria* spp., *Cynodon dactylon*, *Eragrostis curvula* and *Panicum maximum*. In addition, we included four treatments with pasture species without barley and one treatment with conventional barley. The pasture species were sown in May 2015 with irrigation support and mown in June (at barley harvest) and November (prior to barley seeding). Barley was direct drilled into winter dormant pasture stubble in the relay cropped plots. Barley (establishment, yield) and weeds (diversity, density, biomass) were assessed in all cases. Univariate ANOVA and Bonferroni post hoc test were used to test differences according to cropping system using (or not) the different perennial species. Since *Brachiaria* spp. and *P. maximum* did not survive the initial winter, only two pasture species are considered in this study: *C. dactylon* and *E. curvula*. After two years of experimentation, a significant decrease in weed biomass, density (mainly *Polygonum aviculare*) and diversity was observed in relayed cropped plots. The best weed control was obtained with *C. dactylon*. The results of growth and yield of winter barley were similar in all treatments.

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## Legume living mulches for weed management in tropical fruit plantations

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Living mulches increase agroecosystem diversity and provide multiple agronomic and ecological benefits. Weed control and nitrogen (N) fixation are considered important contributions by legume living mulches in organic papaya (*Carica papaya* L.) plantations in Costa Rica. In order to evaluate the effect of living mulches on weed control, nutrient release, and competition with papaya plants, an experiment was carried out on the Atlantic low lands of Costa Rica in 2014. Three legumes, *Vigna radiata*, *Crotalaria spectabilis* and *Pueraria phaseoloides*, were sown 7 weeks after papaya plants were transplanted into the field. The control treatment consisted of the usual weed slashing implemented on the farm. *Vigna radiata* and *C. spectabilis* percent cover was 85% and 90%, respectively, 10 weeks after the cover crops were seeded, whereas *P. phaseoloides* establishment was unsuccessful possibly because heavy rains washed the seeds away. Weed aboveground biomass was 90% and 66% lower in the *V. radiata* and *C. spectabilis* plots, respectively, compared to the control. Dry matter of *V. radiata* and *C. spectabilis* contained 3.1% and 4.9% N, which represented an estimated contribution of 70 and 181 kg N ha<sup>-1</sup>, respectively. There were no differences among treatments of papaya plants height, stem diameter, number of leaves and number of fruits. These results show that legume living mulches are a feasible strategy for weed control and nitrogen fixation during the establishment phase of organic papaya plantations.

## **Evaluation and characterization of propane gas based flaming system for onion (*Allium cepa*)**

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Onion (*Allium cepa*) is an important vegetable crop in Israel. Its leaf morphology and low vigorous, make him a weak competitor with weeds. Moreover, the reduction in herbicide availability due to regulation requires adoption of alternative weed control methods. Propane gas flaming offer an alternative strategy to cope with weeds in onion. Thus, elucidating the advantages and disadvantages of this method is essential for the development of an affective weed management system for onion. Our objectives were to: **(a)** examine the efficacy of local Israeli weed species control by flaming, and **(b)** characterize the time window for safe flaming application in onion. Five weed species were grown and flamed at five propane doses on different phenological stages. To determent the timing for safe use six onion varieties were grown and flamed using a single propane dose at four phenological stages. A 70%-95% reduction in weed biomass was observed at the first phenological stage, compared to control. However, there was no reduction in weed biomass at the later phenological stages. in the pre-emergence stage biomass didn't changed for all onion varieties, while >95% biomass reduction was observed at the flag leaf stage. At the first and second true leaf stages there was a great difference in the tolerance of the tested onion varieties, starting from complete tolerance (0% biomass reduction) and up to completed susceptibility (95% biomass reduction). Our results demonstrates the potential of using flaming approach for weed control in onion and the importance of setting an accurate time window and propane doses for effective and safe weed flaming in onion.

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## **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.agpropane.com/ContentPageWithLeftNav.aspx?id=1916>

### **Inter-row hoeing for weed control in organic spring cereals**

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New camera technology capable of detecting crop rows now makes it possible to employ selective weed control in spring cereals. Cereals are normally grown at 125 mm inter-row spacing but even moderate extension of the spacing can make enough room for automatically steered inter-row hoeing; the technology is being employed on an increasing number of organic farms. However, intra-row weeds, i.e. those growing in the crop lines, are not controlled and increasing the inter-row spacing to  $\geq 250$  mm may cause a yield penalty. The aim of this study was to investigate the interaction between inter-row cultivation, inter-row spacing and nitrogen rate on weed and crop growth. Results encompass four years field experimentation with spring barley and spring wheat including studies on optimal hoe blade configurations for hoeing at narrow inter-row spacing. Weeding effectiveness was generally greater in barley than in wheat but with minor differences between the inter-row spacing studied (125-150-200-250-300 mm). Maintaining the seed rate when increasing inter-row spacing was important for preserving crop yields and in most cases wide inter-row spacing (300 mm) did not yield less than the other spacing. Nitrogen rate only affected crop yields. The traditional 'Ducksfoot' blade was not optimal for inter-row hoeing at small row spacing due to excessive side wards soil movement covering the crop leaves. Two new hoe blade designs performed better in this regard and especially the one design was very stable during operation. Inter-row hoeing for spring cereals is particular promising for weed control at high weed infestation levels, and where many erect and tap-rooted weed species are present.

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## **Do water management strategies may affect competitive ability of semi-dwarf rice cultivars with weeds?**

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Echinochloa species are well-known weeds, with a worldwide distribution. It is well adapted to temperate regions and anaerobic conditions, such as, rice fields and wetlands. In rice, weeds are among the major biotic factors that cause a high decline in productivity. Water management can enhance competitiveness of cultivars against weeds and it could be acceptable cultural control method to farmers through reduce dependency on herbicides. With this research, we evaluated the weed-suppressive effects of water levels and rice cultivars against important rice weeds. The studies comprised of three water levels (low, medium and high), three medium-grain rice cultivars (Ronaldo, Nembo and, Cameo) and three weed levels (weed-free, low and high weed density). Weed-free conditions supported the rice plants to achieve excellent growth and development, and produced highest grain yield, while weedy conditions hampered the growth and productivity of rice cultivars. Water levels had the greatest impact on weeds, rice growth and paddy yield. A standing layer of water (high water level in particular) not only had a highly suppressive effect against weeds but it also helped to improve the growth and productivity of rice cultivars. Deep and shallow water levels respectively helped to increase the leaf area, number of tillers, and dry weight of rice cultivars, particular the Ronaldo. Ronaldo performed better than other cultivars regarding the growth (either under weed-free or weedy conditions) and weed-tolerance. This work concludes that maintaining a water layer in rice field could produce multiple beneficial effects including an improved rice growth and decreased weed growth, subsequently leading to high rice productivity.



**Effect of row spacing on yield and critical time of weed control in corn**Dejan Nedeljkovic<sup>1</sup>, Dragana Bozic<sup>1</sup>, Stevan Knezevic<sup>2</sup>, Sava Vrbnicanin<sup>1</sup><sup>1</sup>University of Belgrade, Faculty of Agriculture, BELGRADE, Serbia<sup>2</sup>Department of Agronomy and Horticulture, University of Nebraska, LINCOLN, United States of America

The adoption of narrow row spacings has primarily been driven by the potential for higher yields in the twin rows systems (TRS) compared to the standard rows systems (SRS). During two vegetation seasons experiments were conducted in southern Banat (Serbia), to determine the critical time of weed control (CTWC) in hand-weeded corn in two corn systems production, SRS (seeding rate 80000 seeds ha<sup>-1</sup>) and TRS (seeding rate 105000 seeds ha<sup>-1</sup>). All treatments were arranged in a split-plot design with three replications. There were two main plots which included: (i) SRS and (ii) TRS. Each main plot divided in two sub-plots, one with PRE herbicide (s-metolachlor + terbutylazine) application (WPHA), and second without PRE herbicide application (WOHA). For each main plot, seven treatments were imposed on the sub-sub-plot experimental units, of which five treatments had weeds growing until: BBCH 13, 16, 19, 34 and 52; and season-long weed-free and season-long weedy treatments. The size of experimental unit was 42 m<sup>2</sup>. Determination of the CTWC in was based on 5% yield loss level. All statistical analysis was performed with R program utilizing the »drc« package.

In general, yield loss increased with increasing duration of weed interference in both corn systems production. CTWC in SRS corn production for 2015 year were at BBCH 12 in treatments WOHA and BBCH 14 in treatments WPHA, while in TRS were BBCH 13 in treatments WOHA and BBCH 14 WPHA. The same experiments were repeated 2016 year in SRS corn production CTWC were at BBCH 11 in treatments WOHA and BBCH 13 WPHA. With TRS production in treatments WOHA CTWC were at BBCH 12, while in treatments WPHA were at BBCH 15. Based on results from CTWC experiments, are less likely to cause yield reductions in TRS compared to SRS production in season with optimal weather conditions.

## **Weed management in cereals with cover crops - do they help or hinder?**

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Sustainable crop production calls for integrated weed management approaches. Cover crops (CCs) have been studied primarily in the context of soil fertility and soil structure but they might also supplement the weed management toolbox, particularly when adapted to long-term control strategies. The challenge in organic cropping is to provide strong weed suppression without severely compromising weed species diversity and crop yields. Several CC species, mainly sown in mixtures, were studied in organically cropped 3-year field experiments in southern Finland. CCs were sown concurrently with spring barley in 2015 and in the same plots with winter wheat in early May 2016. The subsequent effect on weeds and crop yield was studied in spring wheat in 2017. Among the studied legume species, low-growing *Trifolium pratense* L. and *T. repens* L. were more suitable CCs than the tall species *T. incarnatum* L. and *T. resupinatum* L. var. *majus* Boss. Italian ryegrass (*Lolium multiflorum* Lam.) fitted well in mixtures with clovers. White sweet clover (*Melilotus alba* Med.) was highly aggressive both as a CC in 2016 and as a volunteer weed in 2017, particularly in reduced tillage. Managing with CCs and weeds remains questionable in reduced tillage systems. Annual weed species which emerged early in the spring and grew fast and tall were not effectively controlled with CCs. The CC competition against weeds increased towards and after harvest time and therefore more profound studies on weed seed/rhizome suppression are warranted. With an optimal CC mixture, seed rate and sowing time no significant yield losses were detected in a sufficiently tall and dense crop. CC establishment in clay soil in early spring works well for both spring and winter cereals but prevents mechanical weed control operations at later growth stages. The study was part of the PRODIVA project »Crop diversification for better weed management«.

## **The long-term effects of potassium fertilisation on weed control in old permanent pasture**

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Perennial grassland weeds are often believed to have damaging effect in managed pastures. Fertiliser application enables farmers to influence pasture production. Potassium (K) is supposed to be one of the essential elements with specific physiological role in plants. Therefore K is very important for the legume growth and sustainability in permanent pasture.

A long-term fertiliser experiment, which was set up in 1961 on a loamy cultivated soil is continued 47 years. Plots were fertilised with different doses of potassium. Since the experiment was established, plots have been grazed. The focus was estimate botanical composition of the long permanent pasture in relation to suppress perennial weeds by increasing the content of high nutritive value swards. During the experimental period, dry matter (DM) yield was found to be significantly positive affected by potassium application. A regular application of K makes the pasture to maintain varied a good sward amount (legume 21.7-25.5, grass 41.5-47.2%) in total DM yield at fertilisations levels of K 25 and 75 kg ha<sup>-1</sup>. In the first four experimental years (1961-1964), the content of weeds mostly varied irrespective of whether potassium fertiliser was applied or not up to 6.5 % in the total DM yield. However weeds amount significantly decreased at the fertilisations of K during the last four experimental years (2005-2008). The weeds content in non-fertilised treatment ranged up to 39.0 % while the average in the fertilised plots ranged significantly lower between 31.1 and 33.9% for the period (P <0.05). The main species of weeds were *Taraxacum officinale*, *Leontodon autumnalis*, *Achillea millefolium* and others (13.4-16.2, 1.2-8.6, 1.9-3.4 and 12.9-20.3 %, respectively) in the total DM yield. In an older sward, the higher K 50 kg ha<sup>-1</sup> rate exhibited a significant advantage over the smaller rate K 25 kg ha<sup>-1</sup> on herbage nutritive characteristics and weed suppression ability.

### **Study the effect of undersown Persian clover on weeds in rapeseed**

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Adapting integrated weed management approaches and search for new technique is of increasing demand due to development of herbicide resistant weed biotypes and environmental hazardous. As a search for new weed control measure, it was decided to study the effect of undersown Persian clover (*Trifolium resupinatum* L.) on weeds in rapeseed for the first time in Iran. Therefore, a field experiment was conducted in Nahavand region of Iran, where rapeseed is a common crop, during 2016 to 2017. The experiment was consisted of rapeseed sown with 25, 50, 75 and 100% of the recommended seed rates of Persian clover (50 kg/ha), rapeseed under weed free and infested treatments in two regions in a randomized complete block design with four replicates. Wild oat, field bindweed, bristly foxtail and common poppy were the dominant weeds. The results showed that undersown Persian clover caused reduction in density, fresh and dry weight of weeds significantly. Weed dry weight reductions were 12.5, 15.9, 22.4 and 30.3% for rapeseed sown with, respectively 25, 50, 75 and 100% seed rates of Persian clover in one location. In the second location, weed dry weight reductions were 5.4, 7.5, 15.4 and 16.9% for rapeseed sown with, respectively 25, 50, 75 and 100% seed rates of Persian clover. Rapeseed yield and its components were not improved by undersown Persian clover. However, undersowing of Persian clover could be a promising approach to introduce in the cultivation due to its effect on weeds, in addition to its nitrogen fixation quality and possible ecosystem services. The results suggest that the adoption of undersowing of Persian clover can be considered as a complementary component of an integrated and sustainable weed management system in rapeseed due to its effect on weed management and ability to supply several ecosystem services e.g. nitrogen fixation and carbon storage.

**Weed suppression by fall-sown legume and grass cover crop mixtures in organic agriculture**

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Cover crops are used as a tool to preserve the environment and agrobiodiversity, to enhance soil chemical and physical properties, and help in suppressing weeds. A multi-species cover crop mixture was compared with single-species cover crop for weed suppression and soil fertility builder. The study was conducted at the American Farm School, in a clay-loam soil, for three consecutive years. Four species of annual legumes (*Vicia villosa*, *Trifolium incarnatum*, *Vicia faba minor*, *Pisum sativum*) and three grasses (*Triticale*, *Avena sativa*, *Lolium multiflorum*) were used as cover crops and their weed suppressing ability was compared to a bi-culture (vetch & oats), and a single-species grass cover crop treatment (triticale), with a cultivated fallow treatment as a control in a RCB (Randomized Complete Block) Design. The leguminous cover crops were inoculated with the corresponding group of rhizobium inoculum. A UAV (Unmanned Aerial Vehicle) with an infrared camera was used to monitor cover crop growth and canopy development. Number of weeds within experimental plots and their biomass was measured and weed suppression of each cover crop system was estimated. All cover crop systems exhibited a high weed suppressing ability. The multi species cover crop system was shown to be very adaptable, consistently producing a high biomass in all years. In one season, when the cover crops were established late due to weather conditions, the bi-culture was found to significantly increase soil total nitrogen and nitrates, producing a high legume biomass, and contributing significantly higher PAN (Plant Available Nitrogen) than all other cover crop systems studied. The multi-species mixtures have also been found to have a great potential to produce a high biomass. Both the bi-culture and the multi-culture cover crop systems were found to perform better for weed control and soil fertility management, compared to the single species grass cover crops.

## **Mechanical rice transplanting as a tool for weed management in organic rice**

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Weed control is one of the main issues in organic rice cultivation. Rice transplanting can favor rice competition against weeds and allow weed control with inter-row tillage. A two-year project started in 2016 aimed at evaluating the possibility of applying mechanical rice transplanting in Italy. Rice was mechanically transplanted on 66 hectares included in 24 rice farms involved in the study, all located in the Lombardia region. Seedlings were grown in a nursery following the »mat method«, in which about 4,700 rice seeds were sown in a single tray of 60 x 30 cm, filled with a potting mix. About 200 trays were necessary to transplant a single hectare. Trays were sown with nine different rice varieties: Brio, Cammeo, Centauro, Cerere, Ronaldo, Selenio, Spillo, Venere and the hybrid variety Ecco 63. Seedlings were transplanted at 2-3 leaf stage with a self-propelled riding type transplanter, able to transplant 8 rows of rice simultaneously. The transplanting distance between rows was 30 cm, while the spacing between plant hills within the row was 17 cm. The transplanted plant hills consisted of 2 to 6 plants. The transplanting operations were conducted between mid-May and end of June. Weed control was carried out with a prototype rear-mounted inter-row hoeing machine, designed to work on saturated soil. Fields were weeded once, at about 30 days after transplanting. During the season, weed infestation was monitored in three transplanted fields, while rice yield was assessed in all the fields. The growth advantage given to rice by transplanting and the inter-row hoeing were not able to completely suppress weeds. Weed density ranged from 65.4 plants m<sup>-2</sup> (hybrid variety) to 131.2 plants m<sup>-2</sup> (Selenio). Rice yield showed high variability, with values ranging from 2.6 t ha<sup>-1</sup> (Venere) to 6.2 t ha<sup>-1</sup> (hybrid variety Ecco 63).

**Cover crops as mulching to manage weeds in organic rice cultivation**

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Cover crops sowing after rice harvesting has begun to spread in organic rice cultivation in Italy as a practice to improve soil fertility and weed management. A study aimed at evaluating the use of cover crops during winter in organic rice production and to estimate the effect of the technique on weeds and rice yield was carried out at two sites in NW Italy. In both experiments, the following cover crops were compared: *Vicia villosa*, *Lolium multiflorum*, and a mixture of *V. villosa* (40%) and *L. multiflorum* (60%). A plot without cover crops was added as a reference. Cover crops were sown in October and let grow until spring. In May, rice was broadcasted in the standing cover crops and the cover crops were terminated immediately after. Roller-crimping and shredding were compared as termination techniques. After cover crop termination, the plots were flooded for about 10 days in order to start cover crop decay and stimulate the production of phytotoxic compounds that may hamper weed infestation. Afterwards, rice field was dried to favor rice rooting. Rice was then grown organically with continuous flooding. Cover crop density was assessed after establishment, while weed density was assessed before cover crop termination and during rice growing season. Rice yield was also recorded for each plot. Weed density assessed in June showed that in both experiments the control plots were the most infested (> 900 plants m<sup>-2</sup>), while the plots with *L. multiflorum* both crimped or shredded were the less infested (< 60 plants m<sup>-2</sup>), followed by the plots hosting *V. villosa*. Rice yield was higher for *V. villosa* (3.4 t ha<sup>-1</sup>) plots, while lowest for mix plots (1.1 t ha<sup>-1</sup>), regardless the cover crop termination technique.

## **Explanations for *Amaranthus retroflexus* growth suppression by cover crops**

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Many growing cover crops successfully suppress weeds. Weed control can be due to resource competition and/or allelopathy and is often associated to CC biomass development and subsequent shading, but for certain CC other factors might be implicated. We therefore studied the factors responsible for amaranth (*Amaranthus retroflexus*) growth suppression by different cover crops (CC). In field trials with two shading levels amaranth biomass was similar, demonstrating that light interception by CC was not the primary mechanism responsible for growth suppression. Below a threshold of 3 t/ha of CC biomass, amaranth growth suppression was negatively correlated with CC biomass ( $R^2=0.41$ ) and this correlation was influenced by the CC species. Brassicaceae and black oat (*Avena strigosa*) did not follow this relation and effectively controlled amaranth even with a low biomass.

The effects of root interactions between amaranth and CC on amaranth growth were tested in the absence of resource competition under controlled conditions. Buckwheat suppressed growth of different weeds without physical root interactions, probably through allelopathic compounds. Root exudates were obtained from buckwheat (BK), pigweed (P) and a buckwheat/pigweed mixed culture (BK-P). BK-P root exudates inhibited pigweed root growth by 49 %. Metabolomic analyses of root exudates revealed that BK and BK-P had a different metabolic profile, suggesting that buckwheat changes its root exudation in the presence of pigweed indicating heterospecific recognition. Our findings might contribute to the selection of crops with weed suppressive effects for sustainable weed management.



**Survival of wildflower seeds at 42°C as proxy for survival probability in biogas reactors**

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In Germany, maize is the main substrate used in the production of biogas. One alternative to the continuous cultivation of maize is growing wildflower mixtures, designed for use in biogas plants, in the field margins. In previous studies, some wildflower species appeared to have the potential to survive anaerobic digestion at 42°C, thus posing a risk that they may spread when the digestate is used as a fertilizer. Here, we verified these results at the commercial scale. In addition, seed survival in a buffer solution at 42°C was tested in order to establish an inexpensive proxy for the survival probability in anaerobic digestion.

Seeds of *Chenopodium album* L., *Malva alcea* L., and *Melilotus officinalis* (L.) Pall. were exposed to anaerobic digestion at 42°C in a laboratory scale and a commercial biogas reactor for a maximum of 35 days. Both reactors were fed a mixture of maize silage and liquid manure. In addition, seeds were incubated at 42°C in sterile buffer (pH 7) for maximum 36 days. Viability of seeds was checked for a series of exposure times measuring germination and the response to tetrazolium staining. Mean inactivation times were calculated and compared between the incubation in buffer, anaerobic digestion in the laboratory and in the commercial reactor.

For all species the mean inactivation times were highest in the buffer, lower in the laboratory and lowest in the commercial biogas reactor. For *C. album*, this trend was significant, meaning that seeds were inactivated slowest in buffer.

Our results indicated that screening seed survival in buffer at 42°C is an appropriate proxy for the survival probability of wildflower seeds in commercial biogas plants operating at 42°C. This method may facilitate the optimization of wildflower mixtures aiming to prevent the spread of the seeds with the digestate.

## Weed population effects of reduced tillage systems and weed control strategies of silage maize

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Silage maize (*Zea mays*) cultivation is associated with negative impacts on soil and water quality as reduced soil organic matter, nitrate leaching, soil-biota decline, soil compaction and herbicide runoff. Efforts are made to reduce tillage intensity to overcome these negative impacts. Although less intensive tillage reduces cultivation cost directly, longer term effects may contrast, e.g. regarding weed pressure and weed flora, possibly asking for different weed control strategies. In a long-year field experiments, on marine loam soil and running from 2009, conventional inversion tillage is compared with reduced tillage systems: non-inversion tillage and direct seeding, both tested under chemical and mechanical weed control. The monoculture experiment started from a grassland situation. The experiments are set up with a randomised complete block design with 60 m<sup>2</sup> plots. Weed counts in 1.5 m<sup>2</sup> subplots are made yearly 6-7 weeks after sowing, before chemical weed control.

The most important weed species initially present were *Poa annua*, *Stellaria media*, and *Chenopodium album*. Over time *Solanum nigrum* and *Senecio vulgaris* appeared in significant numbers. On average *C. album* and *S. nigrum* are the most abundant species. Conventional tillage shows lower weed numbers than the reduced tillage systems, but weed abundance dynamics vary with year, and with tillage system and weed control strategy. Higher numbers of *S. nigrum* were observed in 2011-2012 and 2015, whereas *C. album* was relatively abundant in 2014 and 2016. At conventional tillage under chemical weed control (ploughing in Spring) *C. album* and *S. nigrum* numbers increased over the years, whereas only *S. nigrum* increased under mechanical control. At non-inversion tillage *C. album* showed an increase both under chemical and mechanical whereas *S. nigrum* levelled or decreased under the respective weed control strategies. At direct seeding *S. nigrum* numbers decreased and *C. album* numbers increased, both under chemical and mechanical control.

**S08-O Herbicide Resistance  
ORAL PRESENTATIONS**

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## **Farmers' perspective on herbicide resistant weeds and application of resistance management strategies in Germany**

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Studies have shown that the uptake of management strategies to prevent or delay herbicide resistance evolution is highly influenced by farmers' personal experience with resistance and perception of the effectiveness of a specific management strategy. Understanding farmers' perception of herbicide resistance issues and factors that drive farmers' adoption of certain resistance management are therefore crucial for the development of sustainable anti-resistance programs. A herbicide resistance survey was conducted in Germany in 2015 to determine farmers' awareness of herbicide resistance and personal experience with resistant weeds, the information sources used to learn about herbicide resistance and the methods employed to confirm resistance in the field. In addition, the application pattern and perception of resistance management strategies by farmers were assessed.

The majority of farmers (88%) was aware of the presence of herbicide resistance cases in Germany and 64% and 50% of the farmers reported that resistant weeds were detected in their county or on their farm, respectively. Resistance management strategies were applied by 87% of the farmers and the strategy of rotating herbicide mode of action within the crop rotation was ranked the most effective strategy (rating of 8.5 on a scale of 1-10) to minimize the evolution of resistant weed populations. Using a hierarchical agglomerative cluster analysis, three clusters of different resistance management approaches used by farmers to minimize the evolution of resistant weed populations were identified and linked to farmers' perception of resistance and farm-management characteristics. When asked about obstacles to adopting resistance management strategies, higher cost was the obstacle most cited by farmers followed by weather and labour intensity/labour costs. Outcomes from this survey may help researchers and crop consultants to increase understanding of farmers' perception of herbicide resistance issues and to develop resistance management programs with high agronomic practicability and acceptance by farmers.

## **A whole-transcriptome approach to unravel non-target-site-based resistance to ALS inhibitors in *Ambrosia artemisiifolia***

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Non-target-site-resistance (NTSR) to herbicides is a major cause for chemical control failure in a number of weeds. Studies investigating NTSR essentially considered grass weeds. Here, we investigated the transcriptomic bases of NTSR to ALS inhibitors in the global, allergenic weed *Ambrosia artemisiifolia*. Our objectives were to establish a transcriptome resource for *A. artemisiifolia* and to use a comparative, quantitative transcriptome sequencing approach (RNA-Seq) to identify candidate NTSR genes in this species.

Using the PacBio sequencing technology that generate long sequence reads, a transcriptome resource was obtained from the apical part of one 4-leaf plant, i.e., the developing tissues where ALS inhibitors exert most of their effect. BUSCO analysis of the 51,243 contigs (110.5 Mb) indicated a completeness of 80.2% for the assembly, with a N50=2,427 nucleotides.

RNA-Seq was performed on 4 pools of three untreated plants each. Plants in one pool were all resistant (4 pools) or sensitive (4 pools) to imazamox, one major ALS inhibitor used against *A. artemisiifolia*. Expression levels of all contigs in the transcriptome database were monitored and compared among pools. Sixty-two annotated candidate NTSR contigs were identified on the basis of a significantly different expression in the resistant pools compared to the sensitive pools. They included 5 P450s, 14 glycosyl-transferases, 3 glutathione-S-transferases, 4 ABC transporters and 36 other various secondary metabolism enzymes. Candidate contig expression was then measured individually in 384 plants which sensitivity to imazamox had previously been characterised. This allowed to check candidate contig link with NTSR to imazamox in order to identify NTSR markers. The direct role of the NTSR markers in resistance needs to be assessed by functional validation, and the mutations at the root of the differences in expression observed remain to be identified.

## Large-scale field epidemiological surveys reveal evolving glyphosate insensitivity in UK populations of *Alopecurus myosuroides*

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*Alopecurus myosuroides* is the predominant weed species affecting arable cropping in the UK, with its impact exacerbated by evolved resistance to multiple herbicides. The low diversity of available herbicides and the high frequency of herbicide resistance has led to an increasing reliance on glyphosate as a means to control this weed. To date, there are no confirmed cases of glyphosate resistance in *A. myosuroides*, though there is precedence for increased glyphosate use in comparable systems to result in the evolution of resistance. This study utilises 89 field-collected UK *A. myosuroides* populations, for which long-term field management histories are available (herbicide applications, crop rotations and cultivation regimes). Using a dose response experiment, we have established the extent of population-level variation in glyphosate sensitivity. Hence, we present an epidemiological analysis that correlates historical management factors and population size with current glyphosate sensitivity, to provide a pre-emptive assessment of putative risk factors for the evolution of glyphosate resistance. The study is novel in taking a proactive approach to glyphosate resistance management, testing the hypothesis that shifts in glyphosate sensitivity may pre-empt evolution of resistance. Results confirm that none of the tested populations currently exhibit resistance to glyphosate at the field rate. Nevertheless, we demonstrate significant variability in glyphosate sensitivity with an approximate 2.5-fold difference in LD<sub>50</sub> between the most and least sensitive populations. Reduced glyphosate sensitivity was strongly predicted by higher glyphosate use, suggesting selection on this trait in the field. Finally, we show that this selective pressure is increasing as glyphosate usage has significantly increased over the surveyed farms. These results highlight the value of a large-scale epidemiological approach to understand the evolution of pesticide resistance, and provides one of the first truly pro-active studies to identify signatures of herbicide selection before a resistance outbreak.

## Can herbicide hormesis act as a driver of weed resistance evolution?

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Plant production is facing a huge challenge in fighting weeds that have evolved resistance to herbicides. Numerous factors have been identified that enhance the risk of resistance evolution and recently, a new aspect has been suggested to have a putative impact on resistance evolution in weeds, namely herbicide hormesis or else low-dose growth stimulation by herbicides. With weed biotypes that are resistant to the applied herbicide, the full regular application rate can directly represent a subtoxic low-dose to these biotypes and, thus, a potential hormetic dose. This does not directly cause a selection pressure on weed populations, but may accelerate resistance evolution by making hormetically enhanced resistant weeds more competitive, more resistant to a second weed control measure, and/or more reproductive.

To investigate the relevance of this phenomenon, two greenhouse experiments were conducted with sensitive and target-site-resistant (TSR) biotypes of *Chenopodium album* (PSII-TSR) and *Alopecurus myosuroides* (ACCCase-TSR) under the following objectives: (1) Do sensitive and resistant weeds differ in the quantitative expression and sustainability of herbicide hormesis?; (2) How is hormesis expressed within a highly heterogeneous, herbicide-resistant field weed population?; (3) Is hormesis induced by a herbicide application during the weed seedling stage sustained until seed set?; and (4) Does herbicide hormesis increase the reproductive output of resistant weeds at rates normally used for weed control?

Results showed that TSR plants treated with realistic herbicide use rates produced up to 51% more biomass than untreated plants and a herbicide application during the seedling stage of *C. album* proved sustainable to increase seed yield of treated TSR plants by up to 32% above the untreated control. A regular herbicide application can, thus, not only select for resistant genotypes, but further hormetically boost selected plants. This may accelerate resistance evolution by making hormetically enhanced resistant weeds more competitive and more reproductive.

## **The role of differentially expressed glutathione-S transferases in flufenacet resistant ryegrass (*Lolium* spp.) populations**

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Herbicides inhibiting the synthesis of very-long-chain fatty acids (VLCFAs, HRAC group K<sub>3</sub>) have been used for decades in weed control and until today only few cases of weed resistance to these compounds have been observed. Therefore, these compounds have become a valuable tool to control multiple-resistant weed populations *e.g.* resistant ryegrass species (*Lolium* spp.). However, some ryegrass populations have developed resistance to the K<sub>3</sub> mode of action in certain restricted areas. In this study three ryegrass populations metabolically resistant to the K<sub>3</sub> herbicide flufenacet from France, USA and Australia have been selected during a screening to study their resistance mechanisms at the molecular and biochemical level. Analytics approaches (HPLC/LCMS-MS) have shown that resistance to flufenacet is mainly conferred by fast detoxification. Differential expression of their total mRNA was analyzed using Illumina sequencing. The largest proportions of differentially overexpressed genes belonged to the enzyme superfamilies glutathione-S transferases (GSTs), glucosyl transferases (GTs) and ABC-transporters. The DNA sequences of selected GSTs, enzymes potentially catalyzing the first detoxifying step in flufenacet metabolism, were analyzed and used for protein overexpression in *E. coli*. The molecular and biochemical characterization of resistance to flufenacet in different *Lolium* spp. populations allows a better understanding of the evolution of metabolic resistance and its relationships to the chemical structure of the herbicides.



***Conyza* spp. glyphosate resistance: an update on the ecology and mechanism of resistance**

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*Conyza* spp. has been reported the most frequent weed species that developed resistance to glyphosate in various parts of the world. This presentation will provide an update of research data from our group regarding the two most important weed species (e.g. *C. canadensis* and *C. bonariensis*). Differences on germination requirements (light, temperature, pre-chilling) were shown in a number of GR vs. GS populations. Overall, there was no apparent fitness penalty in GR populations. Vacuolar glyphosate sequestration was directly associated with the two key ABC transporter genes (M10, M11); environmental conditions (low temperatures, 8°C) reduced the efficiency of vacuolar sequestration and this was associated with a low level of expression in those genes. Finally, it is recently showed an involvement of epigenetic mechanism in glyphosate resistance (differential methylation pattern) between GR vs. GS populations. Better understanding of the ecology and the mechanism of resistance would help to develop strategies to tackle GR weeds and optimize weed control.

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## **Herbicide Detoxification Mechanisms. A Review.**

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Cost effective Integrated Weed Management (IWM) involves combinations of non-chemical and chemical technologies adapted to the crops, the weed diversity, and environmental factors. When poor diversity is present in the cropping system (including herbicide use) weed-herbicide-resistance can evolve and cause significant crop losses worldwide. Among several resistance mechanisms, herbicide detoxification (EMR, enhanced metabolic resistance) can confer resistance to a broad spectrum of chemical classes representing one or several modes of action. Molecular elements involved in herbicide detoxification are still poorly characterized and understood. Our recent data using RNA-Seq transcriptome analyses to identify genes conferring EMR resistant populations in rye-grass and *Amaranthus* populations resistant to different herbicides will be summarized and compared. Among genes overexpressed in the herbicide-resistant plants compared to the sensitive plants, several, including CytP450s, GSTs, and GTs, were validated by genetics (co-segregation with the resistant phenotype), and functionally, by biochemical activity on the herbicide compounds. For some of the characterized genes, structure activity was performed on a range of herbicides representing several chemical classes. In addition data on protein modelling and herbicide docking in the active site of detoxification enzymes will be presented. Not all overexpressed genes co-segregating with the resistance phenotype were found to be able to detoxify the herbicide(s) showing poor activity on a given resistant population. Possible resistance evolution mechanisms will be discussed in comparison to resistance evolution in other organisms, in particular in insects resistant to insecticides.

## **S08-P Herbicide Resistance POSTER PRESENTATIONS**

## Quick and whole-plant pot assay for detection ACCase herbicides resistance in *Avena sterilis* L.

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*Avena sterilis* L. which is described as winter annual grass weed considered as a highly noxious weed and appears to cause wheat crop losses in the world. Our aim for this study was to investigate the acetyl CoA carboxylase inhibitor herbicides resistance on *A. sterilis* as well as compare between different methods used for investigation. Seeds were collected during shedding in May 2016 from plants that survived clodinafop-propargyl and pinoxaden treatment in wheat field. Susceptible population was collected from the site which has never been exposed to herbicides. For whole-plant assay, seeds were sown on pots and placed in greenhouse under the natural condition during the local winter. Quick test experiment was done in growth cabinet; seeds were germinated on 4.5 cm diameter Petri dishes with two layer of filter paper. At the 2-4 leaf stage, herbicides were sprayed using a charged (MATABI) with a fan-type nozzle. Herbicides rates were 0, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 L<sup>-1</sup>ha for clodinafop-propargyl as well as 0, 0.225, 0.45, 0.9, 1.8, 3.6, and 7.2 L<sup>-1</sup>ha for pinoxaden. After 28 day of the treatment dry shoot weight, plant high and level of injury were recorded. Data were analyzed using Sigma Plot 13 and dose response curve were estimated. Resistant index (RI) estimated from population were 4.87-fold for clodinafop-propargyl and 5.03-fold for pinoxaden in pot assay. RI calculated from petri dishes experiment were 5.46-fold and 4.6-fold for clodinafop-propargyl and pinoxaden respectively. Thus, from our result we can conclude that there is cross resistant in wild oat biotype. Petri dishes test was pretty quicker and does not require as much efforts as the pots experiment. Both assays were able to detect and differentiate between resistant and susceptible biotypes in *Avena sterilis*.

**Acknowledgments:** This study was supported by Scientific Research Projects Unit (FDK-2017-8186) of Cukurova University.

**ALS resistant *Apera spica-venti* - growing problem in Lithuania**

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Grass weed control in cereals is a challenge. *Apera spica-venti* (L.) P.Beauv. is a very common and problematic weed not only in Central and East Europe, but in Baltic countries, particularly in Lithuania as well. High spread of this weed was influenced by growing use of minimal soil tillage and absence of crop rotation. Sulfonylurea herbicides are mainly used for grass and broadleaf weed control in winter cereals in spring in Lithuania, but low efficacy against *A. spica-venti* is sometimes reported in commercial fields. Repeated usage of the same active ingredient for weed control is likely the main factor governing decreased herbicide efficacy and increased herbicide resistance in weeds.

Mature seeds of *A. spica-venti* were collected in 2015 - 2016 from farms where herbicide control of the weed was ineffective. Seeds were sown in pots and sprayed at the 3-leaf stage in a glasshouse experiment. Three herbicide modes of action of herbicides were tested in triplicate on 56 biotypes of *A. spica-venti*: ACCase inhibitor (pinoxaden), PSII inhibition (isoproturon), and ALS inhibition (iodosulphuron, sulphosulphuron and pyrosulam). Visual effects and fresh weight were assessed 4 weeks after application to determine efficacy. All *A. spica-venti* populations were sensitive to PSII and ACC herbicides. 36 populations from those tested were resistant to one or more active ingredients of ALS herbicides. In some plots, where one active ingredient of ALS was used for a long time, low efficacy was recorded for another a. i. of the same mode of action. For such fields other mode of action should be used for weed control.

Autumn used herbicides belongs to other modes of action (F,K, N), therefore herbicide use in autumn is high recommended and in the future unavoidable, especially in early sown winter cereals contaminated by *Apera spica-venti*.

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## Physiological Response of *Amaranthus palmeri* Multiple Resistant to Glyphosate and ALS inhibitors

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Glyphosate is a nonselective and systemic herbicide that inhibits the enzyme 5enol-pyruvate-shikimate-3phosphate enzyme synthase (EPSPS) in the biosynthesis pathway of aromatic amino acids in the shikimate pathway. ALS inhibitors are another popular class of herbicides, which inhibit the acetohydroxy acid synthase enzyme also known as acetolactate synthase (ALS) that participates in the branched-chain amino acid biosynthesis pathway. *Amaranthus palmeri* is one of the most important weeds from United States that cause more problems in crops due to its biological characteristics and its difficult management in agroecosystems. Owing to the enormous selection pressure that has been exerted on the populations through the repetitive use of glyphosate and ALS inhibitors, some populations have developed multiple resistance to both types of herbicides. Although glyphosate and ALS inhibitors have different sites of action, previous studies have shown that they share several physiological effects, suggesting that they kill the treated plants in a similar way. The objective of this study was to characterize the physiological response of one population multiple resistant to glyphosate and to ALS inhibitors. To this aim, two populations of *A. palmeri* (one sensitive and other multiple resistant) were grown in hydroponic culture under controlled conditions. They were sprayed with glyphosate or treated with imazamox (ALS inhibitor) through the nutrient solution. Leaves were harvested after 3 days. Carbohydrate, amino acid content and other parameters related to the biosynthetic pathways specifically inhibited by the herbicides were evaluated. Results are discussed in relation to the possible different physiological pattern of the multiple resistant plants compared to sensitive plants.

## Cross resistance patterns of *Echinochloa* spp. populations among rice herbicides

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INIAV, OEIRAS, Portugal

In Portugal, *Echinochloa* species are the major troublesome weeds in rice. One hundred percent of paddy rice area is treated annually with ALS- and ACCase-inhibiting herbicides. Due to high selection pressure, resistance to penoxsulam was confirmed in *E. phyllopogon* in 2014 in Tagus river valley. To analyze the distribution of resistance in other rice producing areas – Mondego and Sado – a screening process was carried out in 2016.

Seed samples were collected from the affected area and tested. The first screening of 16 populations, to assess the sensitivity of *Echinochloa* spp. to penoxsulame was carried out in a growth chamber dose response study (0,01 to 1000 g L<sup>-1</sup>). Root length was assessed 21 DAT and EC<sub>50</sub> values were estimated using non-linear regression. In whole plant bioassays resistance indices (RI=ED<sub>50</sub> R/ ED<sub>50</sub> S) were calculated for penoxsulam and to other ALS- and ACCase –inhibiting herbicides to assess for possible NTSR.

A susceptible population from each region (EC<sub>50</sub> = 0,16 mg L<sup>-1</sup>) was used as reference. For the screening bioassays 50 % of *E. phyllopogon* populations from Sado rice fields (south) were confirmed resistant (R) to penoxsulam (EC50 values 0,66 to 7,01 mg L<sup>-1</sup>). In Mondego rice fields (center) higher values of EC<sub>50</sub> (1,22 to 46,5 mg L<sup>-1</sup>) and more populations were R accounting for 90 % of the total. In dose-response bioassays with whole plant there were no cross resistance to bispiribac-sodium, cyalofope-butyl and profoxydim for all populations.

From 16 populations of *Echinochloa phyllopogon* analysed eleven were confirmed R to penoxsulam with RI ranging from 3,8 to 44,4 in Sado and 5 to 134,5 in Mondego.. Rice fields from Center of Portugal, account for 90 % of resistance cases. There were no cases of cross resistance among ALS and ACCase –inhibiting herbicides, suggesting TSR as the possible mechanism responsible for resistance.

## Investigating the distribution and herbicide sensitivity of UK arable brome weeds

Laura Davies

ADAS, BOXWORTH, United Kingdom

Previous research has identified UK brome weeds at high risk of developing herbicide resistance. Currently, there is little data on the distribution and potential resistance status of problematic brome species in UK arable rotations: *Anisantha sterilis*, *Anisantha diandra*, *Bromus commutatus*, *Bromus hordeaceus*, and *Bromus secalinus*. However, there are indications that populations of *A. sterilis* and *B. secalinus* are increasing and more wide-spread than previously thought. Although no UK populations have been identified as herbicide resistant, there is an increasing incidence of resistant bromes world-wide. A four-year research project is updating knowledge on UK brome weed distribution in arable farming and investigating the range in herbicide sensitivity and potential for resistance evolution.

In summer 2017, an online survey of UK farmers was conducted to update data on brome distribution and control problems. In addition with known sensitive populations, 58 brome seed samples of *A. sterilis*, *A. diandra*, *B. commutatus*, and *B. secalinus* were received for sensitivity testing to two ALS, two ACCase, and glyphosate herbicides.

Brome weeds were present across all cereal growing areas of the UK, with *A. sterilis* the most abundant species. However, correct species identification was low, with 38% of samples received wrongly identified. Increasing presence and poor control of brome weeds were reported by 60% of respondents. Herbicide sensitivity significantly varied between brome populations and species, with surviving individuals at recommended field rate of ALS herbicides and glyphosate, and a range of 50-98% percent reduction fresh weight at full field rate of ALS herbicides.

The distribution of UK brome weeds is wider than previously thought, extending into regions not historically associated with bromes. Herbicide control problems are emerging, with some populations poorly controlled, increasing the risk of resistance.

This ADAS led project is funded by AHDB, and includes Rothamsted Research, BASF, Bayer, Dow AgroSciences, Monsanto, and UPL.



## High-throughput detection of ALS-based resistance in the tetraploid common groundsel (*Senecio vulgaris*)

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The Next Generation Sequencing (NGS) technologies offer tremendous possibilities for detecting and quantifying mutations endowing herbicide resistance in numerous samples. This would greatly assist resistance management by allowing monitoring numerous fields and/or analysing massive numbers of individual weeds. Yet, NGS use for herbicide resistance diagnosis purpose is very slow to emerge. Herein, we assessed the feasibility of herbicide resistance diagnosis using Illumina sequencing that is currently one of the leading NGS technology. Three PCR amplicons encompassing the eight ALS codons crucial for herbicide resistance (122, 197, 205, 376, 377, 574, 653 and 654) were amplified for both *S. vulgaris* ALS genes in each of 96 non-quantified DNA pools. Each pool consisted of DNA crudely extracted from 50 plants collected in one agricultural field where resistance was suspected (i.e., a total of 4,800 individual plants). The 96 pools were analysed in one single Illumina MiSeq run. A total of 20.8 million quality 250 nucleotide-long paired sequence reads were obtained. Mutant ALS alleles were identified in 61 of the 96 pools. Three previously characterised herbicide resistance-endowing mutations (Pro-197-Leu, Pro-197-Ser and/or Pro-197-Thr) were detected on ALS1 and four (Pro-197-Arg, Pro-197-Leu, Pro-197-Ser and/or Pro-197-Thr) on ALS2. To check the accuracy of NGS-based quantification of mutant ALS alleles, all individual plants in 34 of the 96 pools were individually submitted to ALS Sanger sequencing. The 34 pools were selected to represent the range of mutant ALS frequencies and the diversity of alleles identified using NGS. ALS alleles identified by Sanger sequencing were the same as those identified by NGS. Frequencies of mutant ALS alleles detected by NGS and by Sanger sequencing were very highly correlated ( $R^2=0.986$ ). This work demonstrated the feasibility and the reliability of NGS-based detection of mutations endowing herbicide resistance, and the interest of this approach for analysing large numbers of samples.

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## **Old foe, New threat? Elucidation of *Sorghum halepense* response to ACCase-inhibiting herbicides**

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*Sorghum halepense* (Johnsongrass) is a troublesome perennial weed infesting summer crops. Herbicides such as the ACCase inhibitors are considered the best selective means for effective control of this weed in broad-leaved crops. This study was initiated following farmers reports on poor control of *S. halepense* with ACCase-inhibiting herbicides, aiming at understanding the magnitude of the problem and the mechanisms involved. Seeds and rhizomes of 14 *S. halepense* populations were collected from fields where chemical control has failed, and grown under controlled conditions. Dose response experiments conducted indicated that three populations are resistant to fluzifop-p-butyl and two populations are resistant to rimsulfuron. Based on the ED50 values, the fluzifop-p-butyl-resistant populations from Yagur and Kefar Blum were 28 and 40 folds more resistant, respectively, than the sensitive population from Ayanot. The three populations were equally sensitive to clethodim. A field trial was conducted in Kefar-Blum in a carrot field heavily infested with *S. halepense*, confirmed resistance to fluzifop-p-butyl and other FOP herbicides and sensitivity to clethodim and other DIM herbicides. Further studies are in progress in order to understand the molecular basis of this resistance and to explore alternative management practices to control this troublesome weed.

## **False cleavers (*Galium spurium* L.) with target-site resistance to ALS inhibiting herbicides in Greece**

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*Galium spurium* (Rubiaceae) is a frequent troublesome dicotyledonous weed species, usually controlled with ALS inhibitors, synthetic auxins and their mixtures. In 2016, florasulam+2,4-D failed to adequately control this species in cereal fields (Phthiotida, central Greece) prompting the current study. Seeds from three *G. spurium* populations (G2, G3, G4) with possible resistance and one from herbicide-free field borders (G1, reference) were collected. These were evaluated in a pot experiment for possible resistance to ALS inhibitors at 0, N and 4N (N= recommended field dose: florasulam+2,4-D 4.375+210 g/ha, florasulam 6.25 g/ha, mesosulfuron+iodosulfuron 7.5+7.5 g/ha) with four replicates (five plants/replicate). Mecoprop-p (N= 1050 g/ha) was included as a non-ALS chemical control. Herbicides were applied at the four-six whorl stage of foliar development. Six weeks after treatment, plants were visually scored (VS) on a ten-level scale (0=dead, 10=as unsprayed control) and cut at soil level for fresh weight (FW) measurement. Leaf samples from surviving plants with the highest VS at 4N were collected for ALS sequencing. Mecoprop-p provided 100% control of all populations. Mesosulfuron+iodosulfuron at N fully controlled population G1 (100% plants with VS=0-3) but failed even at 4N to control populations G2, G3 (0% dead plants, 15-16% FW reduction) and G4 (15% dead plants, 47% FW reduction). Florasulam+2,4-D at N moderately controlled population G1 (80% plants with VS=0-3, 69% FW reduction) but failed to control populations G2, G3 and G4, even at 4N (0% dead plants, 4-19% FW reduction). Control with florasulam was complete for G1 only at 4N (100% plants with VS=0-3) but failed for all other populations (0% dead plants at 4N). ALS sequencing revealed a Trp<sub>574</sub>Leu mutation in populations G2, G3 and G4, confirming target-site resistance to ALS inhibitors in *G. spurium*, for the first time in Europe. Further work is underway, with dose-response experiments and ALS enzyme activity assays.

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## **Derived Polymorphic Amplified Cleaved Sequence (dPACS): a novel procedure for detecting known SNPs/DIPs**

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Most methods developed for detecting known single nucleotide and deletion-insertion polymorphisms are dependent on sequence conservation around the SNP/DIP and are therefore not suitable for application to heterogeneous organisms. Here we describe a novel, versatile and simple PCR-RFLP procedure baptised 'derived Polymorphic Amplified Cleaved Sequence' (dPACS) for genotyping individual samples. The peculiarity of the method is that it employs a pair of primers that cover the entire fragment to be amplified except for one or few diagnostic bases around the targeted SNP/DIP. The dPACS assay has a number of advantages over other SNP detection methods, including greater opportunities to incorporate mismatches in one or both 35-55 bp primers for creating a restriction site that unambiguously differentiates wild from mutant sequences following PCR-RFLP and horizontal MetaPhor™ gel electrophoresis. Selection of effective restriction enzymes and primers is aided by the newly developed dPACS 1.0 software. It also provides better possibilities for positively detecting multiple allelic variants using a single PCR reaction and different restriction enzymes and for identifying mutations that are located near the end of exons. As PCR within the dPACS assay targets a relatively short DNA fragment, the method is applicable on highly degraded materials. The dPACS procedure designed on one species is highly transferable to other species because the primers encompass the quasi-entire region to be amplified. The dPACS methodology and its associated benefits are exemplified here with the positive detection in up to 24 diverse grass and broadleaf weed species tested, of wild type proline106 of 5-enolpyruvylshikimate-3-phosphate synthase and its serine, threonine and alanine variants that confer resistance to glyphosate, and serine264 and isoleucine2041 which are key target-site determinants for weed sensitivities to some photosystem II and acetyl-CoA carboxylase inhibiting herbicides, respectively.

## **Apera spica-venti biotype from the Czech Republic resistant to three herbicide modes of action**

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Loose silky bent (*Apera spica-venti*) is one of the most troublesome weeds of winter cereals in Central and Eastern Europe and Baltic countries. Over twelve years, the efficacy of herbicides for *A. spica-venti* control has been tested and the resistance evolution has been monitored in the Czech Republic using whole plant assays and subsequent detection of mutations. The first case of multiple resistance to ALS inhibitors (a.i. chlorsulfuron, iodosulfuron, mesosulfuron) and PSII inhibitor (isoproturon) was described in 2005. The biotype with multiple resistance to ALS and ACCase inhibitors with specific gene mutation at position Pro-197-Ser, Pro-197-Thr and Ile-1781-Leu was found in 2015.

In 2016, the first country case of triple resistance to pyroxsulam (ALS), chlorotoluron (PS II) and pinoxaden (ACCcase inhibitors) was found in one population. Individual plants of this biotype survived not only application of sole herbicides but also tank mix and consecutive application of all three active ingredients at 2N rates. The herbicide treatments were applied post-emergent using the chamber sprayer (AVIKO PK3) equipped with a Lurmark 015F80 nozzle that delivered a spray volume of 250 l ha<sup>-1</sup>. Leaves of surviving plants were sampled and pyrosequencing was performed to identify ALS (Pro-197, Trp-574), ACCase (Ile-1781, Trp-2027, Ile-2041, Asp-2078, Gly-2096) and PSII (Val-219, Thr-220, Ala-251, Phe-255, Gly-256, Ser-264, Asn-266, Leu-275) target-site mutations. From 32 analyzed plants, only three plants showed the presence of Pro-197-Thr substitution. No herbicide resistance-endowing ACCase and psbA gene mutations were detected. This result indicates that the resistance is caused by both target-site and based on non-target site mechanisms and an enhanced metabolism might be responsible for resistance in the first place.

## **African biotypes of *Lolium rigidum* resistant to ALS and ACCase inhibiting herbicides**

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*Lolium rigidum* resistance is a wide-spread problem in Australia not much is known about the herbicide resistance in African ryegrass. In 2011, 2013 and 2015 the small pot experiments were conducted to determine the herbicide resistance pattern in 31 ryegrass biotypes with suspected resistance originating from Tunisia and Kenya. Herbicides were applied when plants were at the 3-leaf growth stage using 0.5, 0.75, 1, 2, 5, 10, 20 fold of registered (1N) rates of pyroxsulam (22.5 g a.i. ha<sup>-1</sup>), clodinafop (15.625 g a.i. ha<sup>-1</sup>), iodosulfuron + mesosulfuron-methyl (3.3 + 0.66 g a.i.ha<sup>-1</sup>) and mixtures of halauxifen-methyl (6 g a.i. ha<sup>-1</sup>) + pyroxsulam and clodinafop (20 g a.i. ha<sup>-1</sup>) + pinoxaden (20 g a.i. ha<sup>-1</sup>). Seven resistant populations were selected for further study. Approximately 70 plants from each population were treated with pyroxsulam (1N) and with iodosulfuron + mesosulfuron-methyl (1N) separately. The percentage of surviving plants was determined. Surviving plants (71-95% in individual populations) were analysed and the mutations in most common positions Pro197 and Trp574 detected. Target site resistance to ALS has been studied by polymorphism. The response to all herbicides tested was profoundly biotype-dependent. In general clodinafop solely gave the lowest efficacy in more than 75% of the tested biotypes. But most biotypes responded to clodinafop + pinoxaden. More than 60% of biotypes were resistant to pyroxsulam. When mixed pyroxsulam with halauxifen-methyl, the efficacy increased significantly. Response to iodo + mesosulfuron differed significantly among populations (RF=1.5-50). The partial sequencing revealed polymorphism in two domains at positions Pro197 and/or Trp574 in 90% of surviving individuals. The study confirmed *Lolium rigidum* populations with cross/multiple-resistance pattern in Tunisia and Kenya. Differences between populations were found in their response to tested herbicides and resistance level, too.

### **Estimation of herbicide resistance in Korean paddy fields**

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Herbicide is the most efficient and important weed control tool in many countries. However, it is getting a big problem to develop herbicide resistant weeds in many countries as well. Since the first sulfonylurea-resistant biotype of *Monochoria korsakowii* was reported in Seosan reclaimed paddy fields, 14 herbicide resistant species have developed in Korea. As well as increasing SU-herbicide resistant species, the estimated infested area is also steadily increasing. In order to estimate the increasing trend of infested area we collected paddy soils randomly from 2,278 sites around the country on March of 2017. Recommended rate of imazosulfuron+pyriminobac-methyl (10.5 g a.i. ha<sup>-1</sup>) was treated to the collected soils and counted emerging species, previously known as SU-herbicide resistant, at 21 DAT. Three replicates and control were included. Approximately 527,127 ha, 62.6% of rice cultivated area excluding organic and eco-friendly cultivated area, was estimated to be infested by SU-herbicide resistant weeds. It has been ca. 3.0 fold increased comparing to 176,870 ha of 2012. The largest infested area was Gangwon-do with 81.1% while the least infested area was Choongcheongnam-do with 48%. *Monochoria vaginalis* was distributed the most, with an area of 293,436 ha, followed by *Echinochloa* spp, and *Schoenoplectiella juncooides*. Emerged species other than the known SU-herbicide resistant species will be tested for their herbicide resistance.

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## **Johnsongrass (*Sorghum halepense*) resistance to ACCase inhibiting herbicides in Serbia**

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The resistance of johnsongrass to ACCase inhibitors has been confirmed in the USA, Greece, Italy, Israel and Argentina. Recent information about the poor control of johnsongrass in some sites in north-western Serbia indicates the possible evolution of resistance in the population of this weed to ACCase-inhibiting herbicides. The aim of the study was to confirm the existence of johnsongrass resistance to ACCase inhibitors and its distribution.

Greenhouse trials were conducted in 2017 to determine the resistance in the suspected johnsongrass populations from Bač municipality (western Vojvodina). To determine the distribution of resistance, seeds of 20 johnsongrass populations were collected from fields where poor efficacy of ACCase-inhibiting herbicides had been reported. Seedlings from those populations were subjected to post-emergence use of two rates of fluzifop-p-butyl (1x=120 g ai ha<sup>-1</sup> and 3x). A population is considered as resistant (R) to a herbicide when more than 20% of treated plants survived the recommended herbicide field rate (1x) and highly resistant (RR) when survivors were more than 20% at dose 1x and more than 10% at dose 3x (Sattin et al., 2001). Also, dose-response trials were conducted with a RR population and susceptible standard population (S) to determine the level of resistance to foliar-applied herbicides: fluzifop-P-butyl, fenoxaprop-P-ethyl, haloxyfop-P-methyl, quizalofop-p-ethyl, quizalofop-p-tefuryl, propaquizafop, clethodim and cycloxydim.

Continuous use of some ACCase inhibitors over multiple years in monoculture of soybean in Bač municipality has resulted in the selection of cross resistance in johnsongrass to aryloxyphenoxypropionate herbicides. Of the total of 20 populations, resistance or highly resistance to fluzifop-p-butyl was determined in 13 populations concentrated on a territory of about 10km in diameter. Dose-response bioassays confirmed that the level of resistance as a ratio of R/S based on ED50 (50% fresh biomass reduction) varied between 3.1 (for clethodim and cycloxydim) and 91.2 (for fenoxaprop-P-ethyl).



## Complex *Amaranthus* spp. populations infesting soybean fields under ALS inhibitor selective pressure

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The most common *Amaranthus* species infesting soybean fields in Italy is *A. retroflexus*, however in the last few years several farmers reported infestations of *A. tuberculatus* (syn. *rudis*) that were poorly controlled by ALS inhibitors. Until recently this invasive species could only be found in riparian habitats and none of the stakeholders had reported infestations in cultivated fields. In 2010, the first population of *A. tuberculatus* resistant to acetolactate synthase (ALS) inhibitors was confirmed in north-eastern Italy. The aims of this study were: investigating the resistance status, the cross-resistance pattern and the resistance mechanism involved in *A. tuberculatus*, *A. retroflexus* and *A. hybridus* accessions (15 accessions in total).

A greenhouse whole plant bioassay was performed and plants were treated at the 2-4 leaf stage with thifensulfuron-methyl and imazamox at the field rate (6 g a.i. ha<sup>-1</sup> and 40 g a.i. ha<sup>-1</sup>, respectively) and 3-times the recommended rate. Herbicides were applied using a precision bench sprayer (300 L ha<sup>-1</sup>, 215 kPa, 0.75 m s<sup>-1</sup>) with a boom equipped with three flat-fan hydraulic nozzles (TeeJet, 11002). Survival and visual estimation of the biomass were recorded 4 weeks after treatment. 5 plants per accession (survived at the thifensulfuron-methyl treatment at the field rate) were sampled for genomic DNA extraction and sequencing of the ALS gene.

All *A. hybridus* and *A. tuberculatus* accessions were resistant to both thifensulfuron-methyl and imazamox. However, all *A. retroflexus* accessions were only resistant to thifensulfuron-methyl. The analyses of ALS gene revealed that all plants of *A. hybridus* and *A. tuberculatus* accessions carried the Trp574Leu mutation, whereas plants from *A. retroflexus* accessions carried the Asp376Glu mutation. Within the same field, different *Amaranthus* species have different ALS point mutations. So far all populations of *A. tuberculatus* sampled during a survey of soybean fields proved to be resistant to ALS inhibitors.

## Occurrence and distribution of weeds resistant to ALS-inhibitors in paddy fields of Korea

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Continuous use of acetolactate synthase (ALS) inhibitors has led development of resistance in several rice weed species, including *Echinochloa crus-galli*, *Monochoria vaginalis*, and *Schoenoplectus juncooides*. *Monochoria korsakowii* was the first weed species resistant to herbicide in Korea in 1998. Since then, thirteen weed species were identified as herbicide resistance. In order to identify occurrence and distribution of herbicide resistant weed species in paddy fields of Korea, we collected soil samples from the 264 paddy fields in Chungnam province, one of major rice cultivating area in Korea. The soil samples were mixed with water and kept flooding at 5 cm depth. Field rate of herbicide mixture including imazosulfuron was applied in the soil samples 15 days after watering. The species and number of survived weeds were identified 30 days after herbicide treatment. Total seven weed species were found after the herbicide application; *E. crus-galli*, *M. vaginalis*, *Lindernia dubia*, *Lindernia procumbens*, *Sagittaria trifolia*, *Cyperus difformis*, and *S. juncooides*. The most resistant weed species were *L. dubia*, followed by *M. vaginalis* and *S. juncooides*. At least one plant survived in 128 sampling sites indicating 48% of paddy fields was infested with ALS inhibitor resistant plants. This estimation is very similar to the result (47.6%) of the experiment conducted in 2011. Long term monitoring of herbicide resistant weeds is necessary to make a resistance management strategy in paddy fields of Korea.

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## Studying cytochrome P450-based non-target-site resistance in *Apera spica-venti*

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*Apera spica-venti* is a dominant grass weed species mainly occurring in winter cereals in Central, Northern and Eastern Europe. Probably due to its genetically diversity, increasing numbers of resistance cases towards ALS-inhibiting herbicides are reported in Germany. In this context, target site resistance (TSR) and non-target-site resistance (NTSR) mechanisms are discussed. For *A. spica-venti*, studies especially on NTSR mechanisms are rare. To identify components of NTSR such as cytochrome P450 that might be linked to ALS resistance in *A. spica-venti*, a population with reduced sensitivity to iodosulfuron was selected from a sensitive parental population (A77) by applying half the registered dose rate of iodosulfuron ( $5 \text{ g ha}^{-1}$ ). In a subsequent dose-response experiment, the progeny population (A77-1) was less sensitive to iodosulfuron compared to population A77 ( $RI = 2$ ). Genetic analysis revealed that this sensitivity shift could only partially be attributed to a Pro197Asn substitution being present in individual plants of the A77-1 population. So, we assume that NTSR mechanisms were selected simultaneously. In a further step, individuals lacking TSR were identified by sequencing the full length ALS gene. These plants were cloned and crossed to yield the population A77-1-1. To identify involvement of cytochrome P450s in resistance of the selected NTSR population, A77-1-1 plants were divided into four clones. Three of these clones were subjected to treatments of iodosulfuron ( $20 \text{ g ha}^{-1}$ ), iodosulfuron ( $20 \text{ g ha}^{-1}$ ) + malathion ( $1000 \text{ g ha}^{-1}$ ) or malathion ( $1000 \text{ g ha}^{-1}$ ), respectively. The fourth clone served as an untreated reference. Plants were harvested 24 h after treatment for RNA extraction. Homologous sequences to 6 cytochrome P450s were identified by ACP-PCR so far. Currently, gene identity and differential regulation are under confirmation.

## **RELIUM: developing knowledge and tools for managing herbicide resistant *Lolium* spp. across Europe**

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*RELIUM - Herbicide resistant *Lolium* spp. in climatically and agronomically diverse European countries: from developing quick and reliable detection tools to devising sustainable control strategies* - is a three-year research project (2017-2020) selected by the EU ERA-NET C-IPM (Coordinated Integrated Pest Management in Europe - <http://c-ipm.org/>). Research organisations and universities from Italy, Denmark and Greece are participating in the project.

*Lolium* spp. is a serious weed problem all over Europe and therefore represents a unique opportunity for gaining a better understanding of the evolutionary process leading to herbicide resistance and for developing management strategies. The project aims are monitoring, online mapping, developing innovative detection tools and characterizing (patterns, levels and resistance mechanisms) selected herbicide resistant populations (i.e. to ACCase, ALS and EPSPs inhibitors), as well as devising resistance management strategies for *Lolium* spp. in contrasting agronomic situations. Each participating country will focus on specific tasks and complement each other by exchanging information and plant material. The first step is to produce updated databases and maps of resistant *Lolium* spp. in the three partner countries. The project will then characterize resistant populations and set up quick molecular tests for detection of target-site based and metabolic-related resistance.

Studying *Lolium* spp. populations in this way will help the partners gain insight into the evolutionary process leading to herbicide-resistant populations. Finally, the partners will improve the management of resistant *Lolium* spp. by developing transnational resistance management strategies adapted to national conditions of each participating country. Relevant stakeholders (farmers' organizations, farmers' advisors and national herbicide resistance action groups) will be involved throughout the project in devising resistance management strategies, discussing and disseminating the outcomes of the project. Dissemination will mainly be done through the web. An open-day meeting will be held in each country at the end of the project to present the results.

## Evidence of *Cyperus esculentus* resistant to ALS inhibitors in Italian rice fields

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Yellow nutsedge (*Cyperus esculentus* L.) is a perennial herbaceous member of the sedge family (Cyperaceae). It has a high potential to reproduce vegetatively through tubers which can survive the winter period. It also produces abundant seeds. Among the herbicides available for weed control in Italian rice crop, only two acetolactate synthase (ALS) inhibitors, i.e. halosulfuron and azimsulfuron applied in post-emergence, are effective to control *C. esculentus*. Other chemical options are less effective. The high reliance on ALS inhibitors to control other weed rice species has increased the selection pressure and in recent years rice growers have reported failure to control nutsedge. This study was conducted to confirm the suspected resistant status of two *C. esculentus* populations collected in rice fields from north-western Italy.

Tubers from *C. esculentus* plants that had survived a treatment as well as from a susceptible population were collected. A whole plant bioassay was carried out in a greenhouse. Seedlings were treated at the 3-4 leaf stage with halosulfuron (30 g p.a./ha) and azimsulfuron (22.5 g p.a./ha) at the recommended label rate. Herbicides were applied with recommended surfactants, using a precision bench sprayer delivering 300 L/ha, at a pressure of 215 kPa and a speed of 0.75 m s<sup>-1</sup>, with a boom equipped with three flat-fan hydraulic nozzles (TeeJet<sup>®</sup>, 11002).

Both populations were found to be highly resistant (more than 80 % survival and biomass) to azimsulfuron and cross resistant to halosulfuron. ALS-resistant *C. esculentus* is of particular concern because it is one of the few resistant cases which involves a geophyte species with a high reproductive ability, there are no other chemical options available and it can infest other important summer crops. A careful integrated weed management approach is needed to avoid the spread of resistant tubers from field to field through machinery and agronomic practices.

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## Resistance to terbuthylazine and metamiltron in *Chenopodium album*, *Amaranthus retroflexus* and *Solanum nigrum*

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The purpose of this study was to identify populations with resistance to PSII inhibiting herbicides in broadleaved weeds after 20 years of use of herbicides with newer modes of action. Seeds from 35 putatively resistant populations of *Chenopodium album*, 15 of *Amaranthus retroflexus* and 7 of *Solanum nigrum* were collected from sugar beet, maize, potato and vegetable fields across the Czech Republic in 2016. To characterize the susceptibility to herbicides, small pots assays were performed. Two active ingredients terbuthylazine (750 g a.i./ha) and metamiltron (1400 g a.i./ha) were applied using the chamber sprayer (AVIKO PK3) equipped with a Lurmark 015F80 nozzle that delivered a spray volume of 300 l ha<sup>-1</sup>.

We detected two biotypes of *C. album* and one biotype of *S. nigrum* that survived a treatment by both tested herbicides. Three other biotypes of *C. album* were resistant only to metamiltron. Four out of fifteen tested biotypes of *A. retroflexus* were resistant to both metamiltron and terbuthylazine whilst nine biotypes were considered as weak, medium or highly resistant to metamiltron only. Sequencing of the *psbA* target gene identified the Ser 264 to Gly mutation in 90-100% of all analysed plant samples which survived the terbuthylazine treatment. The *A. retroflexus* that survived the metamiltron treatment only did not contain the S264G mutation suggesting other potential target site and non-target site mechanisms in these samples.

## **Germination and development of susceptible and herbicide-resistant biotypes of *Apera spica-venti* (L.) P.Beauv.**

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Silky bentgrass (*Apera spica-venti* (L.) P.Beauv.) is one of the most troublesome grass-weeds in Poland. There are also several herbicide-resistant biotypes of this weed in the country. The timing of emergence as well as the development of different populations of silky bentgrass is crucial for their competitive abilities and their response to the herbicides. For this reason the laboratory and pot experiments were established to assess the germination and development of resistant and susceptible populations of *A. spica-venti*.

We studied six resistant and one susceptible populations of *A. spica-venti*. Prior all the experiments seeds were stored in the cool conditions ( $\pm 3$  °C) in darkness. Their germination was performed in the growth cabinet in the three constant temperatures: 8, 18 and 28 °C (10/14 photoperiod) and in the three terms: May 2017, October 2017 and March 2018. It was shown, that the seeds display a seasonal pattern of germination, with a higher germination in October, as compared to May. Also, there is an interpopulation difference in the germination index between both resistant and susceptible populations.

In February/March 2018 the development of silky bentgrass populations in the presence /absence of herbicide (sulphosulphuron) will be studied. The pot experiment will be set up in pots filled up with a top layer of three different soils: degraded chernozem, sandy brown soil and loamy brown soil. The development of silky bentgrass plants will be assessed in the equal time intervals, using BBCH scale. Also their biomass accumulation as well as the seed production will be measured at the maturation phase.

## **Preventing glyphosate resistance in the UK: a practical approach**

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Current changes in usage patterns in the UK have increased the risk of glyphosate resistance development. An over-reliance on a limited group of herbicide modes of action has accelerated the development of herbicide-resistant grass weeds, particularly black-grass (*Alopecurus myosuroides*) and Italian rye-grass (*Lolium multiflorum*). This has been mainly due to a lack of new herbicides, regulatory changes, a limited crop rotation and the under-exploitation of cultural control practices. The main threat is in annual arable crops where glyphosate provides a key role in managing grass-weeds which have developed resistance to selective herbicides.

A five-year research project is currently underway to provide information to develop more robust guidelines to prevent glyphosate resistance, in particular quantifying the four key principles: prevent survivors, maximise efficacy, use alternatives and monitor success. The outcomes will fill data gaps identified by the UK Weed Resistance Action Group (WRAG) Guidelines (2015).

The research investigates the two key risk periods of glyphosate application: (1) Stubbles/pre-drilling when multiple applications are applied and (2) Between crop rows when application is to larger plants with no further risk mitigation. A combination of field and container-based methods are included.

Three field trials were completed in 2016-17. Results support previous container experiments, confirming that the most effective stale seed bed glyphosate timing is at a black-grass growth stage of 2-3 leaves, at a glyphosate rate of >540g a.i., but certainly before tillering (>GS21). Higher glyphosate rates (1080g a.i.) on larger plants can be effective, but the resistance risk is higher if some black-grass plants survive this treatment. Three further field trials were sown in autumn 2017 so results from two seasons will be compared.

Data will provide greater precision and improved evidence to retain effective use of glyphosate for grassweed control in UK arable rotations supporting future management guidelines.



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### **Glufosinate-Resistant rigid ryegrass (*Lolium rigidum* Gaud.) in Greece and its effective management**

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The present study was conducted further to recent complain by several Greek farmers on the reduced susceptibility of *Lolium* spp. to glufosinate in perennial crops. The main objectives of this study were to determine the potential occurrence of glufosinate-resistant populations of rigid ryegrass in several regions of Greece, as well as, to evaluate the effect of the weed growth stage on glufosinate efficacy and suggest alternative herbicides for the effective control of the potentially resistant populations. Fifty Greek populations of rigid ryegrass, sampled from seven prefectures, were studied under controlled conditions. After the initial screening, specific populations were selected and dose–response experiments were conducted with several rates of glufosinate (0.0, 0.098, 0.187, 0.375, 0.75, 1.5, 3.0, and 6.0 kg ai ha<sup>-1</sup>). The recommended rate of glufosinate is 0.75 kg ai ha<sup>-1</sup>. Glufosinate resistance was found in some of the populations with various resistance levels (resistance indices ranged from 3.08 to 7.38), while our results were further confirmed by different ammonia accumulation between the populations after the treatment with glufosinate. Our results also revealed a different susceptibility of the glufosinate resistant populations at different growth stages and therefore it is suggested as an important factor to be taken into account and along with several alternative herbicides could also reduce the selection pressure and achieve adequate control.

## **Inheritance of tribenuron resistance traits in *Tripleurospermum perforatum***

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To determine the inheritance of the acetolactate synthase (ALS) resistance trait in *Tripleurospermum perforatum* from Germany, crosses were made between a tribenuron resistant (R) with an identified target site resistance (Pro-197-Gln) and susceptible (S) population. Seed progeny was collected separately from both parental plants (S and R) generating F1 maternal R (R-F1) and reciprocal F1 maternal S (S-F1) populations. »Pseudo-F2« populations were generated by performing F1 pair-crosses with tribenuron-resistant plants from individual R-F1 and S-F1 populations because selfing is not possible with *T. perforatum*. To test the segregation of tribenuron-resistant traits, F1 individuals were crossed with an individual from the S parent to create backcross (BC) populations. F1, F2 and BC populations were assessed for tribenuron resistance in dose response bioassays using R and S population as references. The inheritance of resistance to chemically dissimilar herbicides (cross-resistance) was evaluated. In addition, surviving plants were tested for mutation at position Pro-197 by pyrosequencing to allow identification of the number of resistant alleles in each plant. The segregation of phenotypic tribenuron resistance in F1 populations was analysed using genetic inheritance models involving one or two loci. Based on dose response analyses, F1 populations were not statistically different from each other, more similar to the resistant parent, and statistically different from the susceptible parent, consistent with nuclear control of the trait and dominance to incomplete dominance of resistance. All surviving F1 plants carried the Pro197Ser substitution. Pyrosequencing data revealed that the frequency of resistant alleles varied within the populations indicating that tribenuron resistance in the tested *T. perforatum* biotype is governed by two isoforms of the ALS gene as both S and R populations were diploid. Data on resistance level and mutation frequency in F2 and BC population are currently analysed.

### **Weed resistance investigations in Latvia**

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Increasing numbers of winter wheat farmers have complained about poor or no control of *Apera spica-venti* (APESV) and *Stellaria media* (STEME) where ALS herbicides have been used intensively. Seeds of APESV and STEME were collected for glasshouse bioassay analysis from fields where ALS herbicides (HRAC, B group) had been used intensively for a long period and poor weed control was observed. Plants grown from the collected seeds were treated with herbicides with different modes of action (MoA) at appropriate timings. Since 2014 samples of young plants were also tested in the laboratory for target-site-resistance (TSR) and enhanced metabolic resistance (EMR) to herbicides from the ALS and ACCase groups. Since 2010 the major observed problem was APESV resistance to ALS inhibitors. In the last three years APESV showed TSR and EMR to ACCase inhibitors (HRAC A group) too, especially from fields with a minimal tillage system and an increasing seed bank in the soil. These herbicides had been applied in spring, but weather conditions during early spring were not favourable for application: night frosts, dry weather, and too late application for APESV. This resulted in poor control and increased selection of resistant plants. STEME green leaves samples also showed TSR to ALS inhibitors. The glasshouse bioassays revealed that all of the STEME samples were totally controlled by pre-emergence herbicides. The worst case was observed in a sample taken from a field where winter wheat was repeatedly cultivated during the past 4 years, and could not be controlled by any of the tested ALS-inhibiting herbicides. This herbicide resistance situation is typical for most farms in Latvia where intensive ALS herbicide usage has been practised with limited crop rotation. Adoption of Integrated Weed Management and better information about herbicide MoA is seen as the best approach to limit the increase of herbicide resistance.

**S09-O Weed Biology  
ORAL PRESENTATIONS**

### **The response of weed species to water stress**

Delphine Moreau, Hugues Busset, Annick Matejicek, Camille Perrot, Nathalie Colbach  
INRA, DIJON, France

Reducing herbicide use requires identifying alternative strategies to regulate weeds. Using crop species that are more competitive for resources than problematic weed species is one lever, but requires a better understanding of weed response to water stress. The present study aimed to quantify the response of three weed species to water stress. In a greenhouse experiment, *Alopecurus myosuroides*, *Amaranthus hybridus* and *Abutilon theophrasti* were grown at 4-5 water regimes, ranging from 75 to 20% of the water holding capacity. Plants were grown individually in pots. Each pot was automatically weighted three times per day, and watered whenever necessary to reach the targeted water regime. A nutrient-rich solution was used. The lower water regimes probably concurred with lower nutrient levels, in line with what is usually going on in agricultural fields. Seven weeks after germination, plants were sampled. For the three species, leaf, stem and root biomasses were affected by the water regimes. The intensity of the response to water stress differed among species, with *A. theophrasti* being the most sensitive and *A. hybridus* the least sensitive species. For plant leaf area, the ranking of plant species for the sensitivity to water regime was similar to the ranking for the potential plant leaf area (i.e. leaf area at 75 % of the water holding capacity). We will identify mathematical equations accounting for the response of plant growth and biomass allocation to water stress. The aim is to identify generic equations, i.e. valid for a wide range of annual species. They will be included in the FlorSys model which simulates weed dynamics and crop canopy growth in virtual fields over the years. The final aim is to use the completed model to identify sustainable weed management strategies that are robust to climatic hazards. Funding: INRA, CoSAC project (ANR-15-CE18-0007), ReMIX (EU-H2020-727217).

## **Characterization of resistance to sunflower broomrape (*Orobanche cumana* W.) in sunflower (*Helianthus annuus* L.)**

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*Orobanche* spp. (broomrapes) are chlorophyll-lacking obligate root parasites, drawing all required water and nutrition from the host. Sunflower broomrape (*Orobanche cumana* Wallr.) is regarded as one of the most important production constraints of sunflower in many countries in Europe and the Mediterranean region. Breeding for resistance is the most effective and beneficial method to manage sunflower broomrape infestation. However, the resistance of new cultivars is often overcome. Therefore a better knowledge of the mechanisms responsible for resistance to parasitic plants is necessary to improve the production of crops with long-lasting resistance. The aim of this study was to elucidate the broomrape resistance mechanism of the sunflower variety 'EMEK3' (a confectionary sunflower hybrid with high and long-term resistance to sunflower's broomrape). Observations of host-parasite interactions along with histological sections of incompatible interaction of *O. cumana* on the resistant variety 'EMEK3' showed that the parasite intrusive cells are stopped in the host cortex, during the penetration attempt, and before reaching the endodermis, indicating a »Pre-Haustorial« mechanism of resistance. Total RNA was extracted from 'EMEK3' roots and from roots bulks of other *O. cumana* resistant and susceptible sunflower lines during the resistance response. A comparative RNA-sequencing identified differentially expressed genes (DEG) associated with the resistance mechanism. The preliminary results of the DEG analysis revealed 3 differentially expressed genes communal to the resistant bulk and 'EMEK 3':  $\beta$ -1,3-endoglucanase,  $\beta$ -glucanase 40-like and ethylene-responsive transcription factor 4-like. These genes are known as PR (Pathogen Related) in other plant species. To conclude, according to the present results, we can presume that the resistant mechanism of 'EMEK 3' is based on the lack of capability of the broomrape to intrude the host root tissue thus the resistance response constitute a physical barrier to prevent parasite penetration.

## Seed persistence and changes in seed coat colouration with time spent in soil

Hana Foffová

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Viability of seeds and their persistence in soil is one of the most important characters in plant ecology, and manipulating it shall be one of the targets of effective weed control. This work is focused on changes in seed viability and seed coat colouration of 26 species with time spent in soil (20 cm depth) for up to 8 years in the soil. The species included in this study were mostly arable weeds. Seed viability was assessed by means of a germination test under constant conditions, and a crush test. Samples were excavated every year, which allowed for modelling the course of change in seed viability with time by means of a logistic regression and estimation of time at which 50 % and 5 % of seed population was still persistent ( $PT_{50}$  a  $PT_{05}$ ). Seed persistence was markedly different among the study seeds, also in seeds of the same family.  $PT_{50}$  viability exceeded 10 years in *Geum urbanum*, *Thlaspi arvense* and *Urtica dioica*. On the contrary, the ones with the shortest survival in soil were *Chenopodium glaucum*, *Campanula trachelium* and *Atriplex sagittata*, which did not survive longer than 2 years ( $PT_{05}$ ) under the experimental conditions of this study. During the course of time, the seeds deteriorated (decomposition and chemical changes), which resulted to changes in the seed coat colouration. Knowledge on these changes may facilitate seed identification from soil samples. The colour of the seed coat was determined by means of image analyses in RGB spectrum. Significant changes in at least one component of the RGB spectrum were found in 17 species. This study contributed to the understanding of changes in weed seed characters due to burial. Supported by the CSF grants 14-02773S and 17-00043S.

## **Are *Striga* spp. and low soil fertility, two sides of the same coin?**

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Infestation by parasitic weeds of the genus *Striga* is often associated with low soil fertility, and both are regarded important crop production constraints in Africa. Despite numerous studies focusing on this association, conclusive evidence for causality between the two is lacking. We conducted a systematic literature search and hypothesized that (H1) *Striga* spp. problems are indicators of poor soil fertility, (H2) soil fertility affects the success of different *Striga* life-cycle stages and (H3) soil fertility determines the extent of *Striga* effects on host-plant performance. The literature search resulted in 124 relevant studies, 82 of which were hypothesis-driven. In 39 studies it was suggested that *Striga* is not found on rich soils or can be reduced by fertilizers (H1.1), however evidence was only provided in 14 papers. The sub-hypothesis that *Striga* is more abundant on poor soils (H1.2) was mentioned by 20 studies, but none of them provided evidence. Out of nine studies, seven confirmed that improved fertility reduces *Striga* germination through decreased host-root exudate production (H2.1). The sub-hypothesis that fertilizers improve crop yields under *Striga*-infested conditions (H3.1) was presented in 19 studies; nine of them affirmed it and one proved it wrong. Four of five studies proved that soil fertility positively affects infected hosts through improved photosynthesis (H3.2), and an equal number proved soil fertility increases infected host biomass and nutrient content (H3.3). Nitrogen levels were inversely related to *Striga* spp. infection levels in 29 studies, while nine studies showed no effect. For phosphorus, ten studies observed such relation and four did not. Literature does not provide convincing evidence that *Striga* spp. are restricted to poor soils, but nitrogen and phosphorus fertilizers were found to improve yields of infected crops and reduce infection levels. Apart from lowering germination-triggering host-root exudate production, mechanisms causing this reduction are still not well understood.



## **The black box of genotype/phenotype variability and its effects on plant fitness**

Henri Darmency  
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To answer the question of anticipating weed outbreaks, researchers use (or should use) population growth rate ( $\lambda$ ) to model demographic evolution. Growth rate is depending of the most frequent genotypes in the population. Not all the plants are equivalent: besides genetics, environmental variables increase the phenotype diversity. To what extent the phenotype shapes the relative fitness of different genotypes? Fitness is the plant contribution to the next generation; it is most often evaluated through progeny size. However, in many studies, this is done regardless of the seed origin and whether the progeny would germinate or not under appropriate conditions and timing to grow as adult plants on further years. In order to estimate how phenotypes interact with genotypes and how fitness is expressed, an experiment was set up with the autogamous *Setaria viridis*. Physiological difference, date of emergence and plant density were chosen to shape phenotypes.

A genotype suffering photosynthesis deficiency was compared to another genotype, thus anticipating different fitness according to previous studies. Seeds were multiplied on year  $n$ , planted in between maize rows and selected for simultaneous emergence with maize or ten days later on year  $n+1$ . Planting design was either isolated plant, mixed genotype pair, or mixed pair plus ten additional plants of one or the other genotype in the same patch. There was seven replicates each treatment combination. Superficial cultivation was carried out after harvest and the field was kept without crop the next years. Date and amount of emerged seedlings, each genotype being identified by collar colour, were recorded the following years  $n+2$  and  $n+3$ . Effects of date and planting design variations on progeny sizes and emergence timing of the two genotypes are discussed. This experiment offers the opportunity for reframing the study of fitness.

### **Facultative or obligate parasitism - does it make a difference?**

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Parasitic weeds are usually classified into three major categories. A first category is composed of facultative parasitic weeds that are able to complete their life cycle in the absence of a host. Parasitic weeds that cannot do without a host are referred to as obligate and include two important classes. Species that contain chlorophyll are named obligate hemi-parasitic weeds, whereas those that do not produce chlorophyll, and are thus completely reliant on their host, are referred to as obligate holo-parasitic weeds. Most of the parasitic weeds that thrive in sub-Saharan Africa (SSA) belong to the class of obligate hemi-parasitic weeds. More recently a facultative parasitic weed, *Rhamphicarpa fistulosa*, has rapidly developed into a major problem in lowland rain-fed rice production systems of SSA. The question that was posed in this study is whether the opportunistic life-history strategy of *R. fistulosa* is better suited to thrive as a parasitic weed in this specific ecosystem. And additionally, whether this advantage is related to specific life-cycle traits or to circumstances specific for this ecosystem. To this end, two life-cycle stage weed population models were developed, that were exactly identical, except for their germination response. In the 'obligate' model seeds only germinated in the presence of a host, whereas in the 'facultative' model germination was independent of host plant presence. Weed seed production in the absence of a host was however substantially lower than in connection with a host. Model analysis revealed the superiority of the facultative life-cycle strategy, which was shown to be mainly due to the short longevity of the weed seeds. As seed longevity is a combined result of species characteristics and the alternate wetting and drying of the lowland soil, it is the combination of species and environment that is responsible for the superiority of one of the two strategies.

## Emergence of different *Echinochloa crus-galli* populations along Europe and the Middle East

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*Echinochloa crus-galli* is a highly competitive annual weed distributed across a wide climatic gradient. Appropriate timing is important for successive control, therefore knowledge about the emergence characteristics can be useful for a decision support system. Emergence of *E. crus-galli* plants was studied in terms of the common experiment run by the EWRS working group Germination and Early Growth. Seeds from two common populations (Italy and Norway) and 1-3 local populations were sown in 25 cm pots at each of the study sites to monitor emergence. Sowing was done in October-November 2015 and destructive counts were performed every 1-7 days, depending on the site. Soil disturbance with adding fertilizer was performed in March-April 2016. Time after soil disturbance required for the plants of different populations to reach 50% of emergence (T50), the maximum emergence rate (Erate) and the maximum emergence number (Emax) were analysed based on a log-logistic model. For the Italian and Norwegian common populations, interactions between site and population factors were found for Erate and Emax, meaning that these populations behave differently in terms of these two parameters. Norwegian population emerged more and faster in higher latitudes and the Italian one in lower latitudes. For T50, it could be concluded that the Norwegian common population emerged significantly earlier than the Italian one. With respect to the local populations, differences appeared mainly when the habitat of origin varied (crop where the seeds were collected), being those from maize the most similar ones between each other. Usually local populations behaved similar to the nearest, in latitude, common population. These results suggest that a model for the emergence of *E. crus-galli* could describe properly most populations, but not all, and that populations from different latitudes can have different temperature and humidity thresholds.

**S09-P Weed Biology**  
**POSTER PRESENTATIONS**

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## **Seed production and seed shattering of black-grass (*Alopecurus myosuroides*) in winter wheat**

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Black-grass (*Alopecurus myosuroides*) has a high reproductive capacity, and herbicide-resistant black-grass has become an increasing problem in autumn-sown crops in northern Europe. There is a need to become less dependent of herbicides and develop new ways of reducing the weed infestation in arable fields. One way to reduce weed infestation could be to destroy or collect weed seeds harvested together with the crop with a combine harvester. The project aimed to estimate how large a proportion of the total black-grass seed production in the growing season a combine harvester potentially would be able to collect. We studied the seed production and the seed shattering of black-grass in a winter wheat field in Denmark in 2017. We recorded the time the matured seeds started to shatter and the number of seeds shattered during the growing season. Black-grass plants were surrounded by a trap comprised of a porous plastic net before flowering. We started counting seeds when plants had shattered some seeds in half of the traps. Then seeds were collected weekly from the traps until wheat harvest. The number of shattered seeds as well as their germination ability was determined. At harvest time we recorded the number of seeds remaining on the black-grass plants. Few seeds were shattered in early July. The number of shattered seeds changed significantly during the season and the highest number of seeds shattered in early August. About 70% of the total seed produced was shattered before harvest. The experiment will be repeated in 2018.

## **Metabolic and biochemical aspects of interaction between species of carrots to root parasite *Orobanche*.**

Sewar Emran

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Broomrapes (*Phelipanche* and *Orobanche* spp.) belong to the Orobanchaceae family. They are chlorophyll lacking root parasite and that parasitize members of many botanic families, and cause yield reduction to a wide variety of agricultural crops in Israel and abroad. The seeds of Orobanchaceae germinate in response to germination stimulants known as strigolactones, presents in the rhizosphere of host plants. Strigolactones are derive from carotenoids via a pathway involving the carotenoid cleavage dioxygenases CCD7, CCD8 and beta-carotene isomerase (D27). In addition, they are required to effect attachment of the germinated seeds to the roots of the host plants via a specialized organ, the haustorium. The haustorium provides them with the ability to extract water and nutrients from their hosts. In our study, we investigate the genetic and essential metabolic relationship between carrots phenotypes and *P. aegyptiaca*. Seventeen genotypes of carrot were sown in 2-liter pots and grown in a greenhouse for six months. Quantification of carotenoids done through a specific extraction runs on the high-performance liquid chromatography (HPLC). In order to obtain further information about the strigolactones pathway and the connection of *Phelipanche* to the carrots, the expression pattern of *Daucus carota* DcCCD7, DcCCD8 and DcD27 genes was examined in seven different colored cultivars including wild type of carrots, as well as in the *P. aegyptiaca* by quantitative RT-PCR. HPLC analysis of carotenoids in the carrot roots showed decreased levels of accumulations in the infested carrot roots, compared to the control experiment. Expression analysis of DcCCD7, DcCCD8 and DcD27 genes showed significant variation in their transcript levels in the different colored carrot genotypes. Our results suggest an important role of the Strigolactones associated genes (DcCCD7, DcCCD8 and DcD27) and in interaction between hosts to the parasite.

### **The influence of high water level in soil on weed emergence**

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In recent years, we can observe problems with standing water on arable land. The subsoil is compacted as a result of repeated heavy machinery movement on the field. Under such conditions, water is replacing air in the soil pores. Plant species that are not adapted to high moisture decrease their germination and emergence. We tested the ability of arable weed species to emerge when the soil environment is influenced by high water content.

In 2016 we established pot trial focused on the influence of water level in the soil on the emergence of arable weeds from the soil seedbank. Four different soil samples were tested, two from Prague – Suchdol - experimental field of CULS (S1), arable field (S2), and two from Nedomice (central Bohemia) – home garden (N1), arable field (N2). 3 treatments with different water levels in four replicates were established: 1. Standard watering (optimum soil moisture, no standing water); 2. water level 6 cm below the soil surface; 3. water level 2 cm below the soil surface.

33 weed species emerging from the soil seed bank were identified. *Echinochloa crus-galli* was dominant species in N2 pots, *Galinsoga parviflora* in N1 and *Chenopodium album* in S1 and S2. In total, we found 1130 individuals, 582 of them were found in treatment no. 1; 433 in treatment no. 2 and 115 in treatment no. 3. When standard watering is considered as 100 % emergence, treatment with water level 6 cm below soil surface was showing 74 % emergence and treatment with 2 cm below soil surface 20 % emergence only.

There were little or no differences between standard watering and water level 6 cm below soil surface. Water level 2 cm below soil surface decreased the emergence of weeds down to 66 % (S1) – 9 % (N1).

## Characterization of the transcriptome during seed dormancy cycling in *Monochoria vaginalis*

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Dormancy cycling is important seed character for weed species to germinate under favorable environment for seedling establishment. Ecological aspects for dormancy cycling have been studied in many weed species, but molecular aspects of dormancy cycling are not clear, which leads to the question of whether different dormant states, such as primary and secondary, are fundamentally similar. In this study, I identified transcriptome during dormancy cycling in a summer annual weed, *Monochoria vaginalis*.

*Monochoria vaginalis* is a serious weed in rice paddy fields in Asia. Microarrays were used for a global transcript analysis of *M. vaginalis* seeds retrieved from a rice paddy environment. The microarrays with 42,378 probes were designed using Ion PGM next generation RNA sequencing data. I also conducted RNA-seq using HiSeq4000, and the reads were used as a transcriptome catalog. The seeds were buried in February 2009 in a rice paddy environment to simulate seed behavior in the soil seed bank and were retrieved every other month from April 2009 to December 2010. We evaluated degree of dormancy by performing germination assays at 25/15C with a 8h light/16h dark daily cycle. Depth of dormancy declined from late winter, then increased from late summer, similar to other summer annual weeds. Microarray analyses revealed that primary dormant seeds had a different type of transcriptome profile than secondary dormant seeds and secondary dormant seeds in 2009 also had a different type of transcriptome profile compared to secondary dormant seeds in 2010. These results indicate that different dormant states are likely to be regulated by different mechanisms. Genes down-regulated in shallow dormant seeds had high homology with *LEA* and *HSP* of *Arabidopsis thaliana*. Up-regulated genes in germinating *Arabidopsis thaliana* seeds, such as *Cyt c* and *SAHH*, were up-regulated in shallow dormant seeds, but down-regulated in deep dormant seeds.



### **Should we be concerned about *Cyperus esculentus* spread via seeds in Switzerland?**

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Yellow nutsedge (*Cyperus esculentus* L.) is an invasive weed in Switzerland. It reproduces and spreads mainly via tubers, but also inflorescences are formed and seeds are produced. However, germination rates of Swiss *C. esculentus* seeds under field conditions are unknown. Further, it is unknown whether *C. esculentus* seedlings can establish and produce tubers under field conditions in Switzerland. These 2 aspects were addressed in this study.

2 populations originating from self-pollination (PSP) from Southern Switzerland and 2 populations from cross-pollination (PCP) between parents from Southern Switzerland and from the Swiss plateau were included in the experiment. Seeds of these populations were sown and cultivated under field conditions at 3 sites in Switzerland in spring 2017. There was no competition from other plants. Site 1 and 2 were irrigated, site 3 was only rainfed. At site 1 and 3 the populations were cultivated in field soil, at site 2 in substrate. Germination rate and tuber production after one season were determined. No seedlings emerged at the rainfed site. Whereas 5 weeks after sowing, germination rates of PSP were 7% at site 1, and 16% at site 2. Germination rates of PCP were 18% and 30% correspondingly. Tubers were produced at both sites by all populations. At site 2, tuber production was similar for the 4 populations: 2 tubers were produced per 10 seeds. At site 1 the PCP produced 17, the PSP 6 tubers per 10 seeds.

Under favorable field conditions Swiss *C. esculentus* seeds germinate. The established plants produce tubers within one season. Seeds originating from cross-pollination tend to have a higher germination rate and might produce more tubers. This is of high relevance as cross-pollination between clones originating from different regions are expected to occur in Swiss fields soon due to the rapid spread of this weed.

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## **Distribution, Germination Characteristics, and Management of Common Groundsel (*Senecio vulgaris*) in Korea**

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*Senecio vulgaris* threaten winter crop cultivation as an exotic and winter weed in Korea recently. As the result of distribution analysis, *S. vulgaris* was widely distributed in Korean cropland around winter crop producing area. The germination was good at 20~30°C and optimum germination temperature was 20/15°C (day/night). More than 4 cm burial depth, the seeds hardly emerged. Linuron, linuron + thiobencarb, and S-metholachlor + thiobencarb showed very good herbicidal effect to *S. vulgaris* through agar and soil assay. For effective and efficient management of *S. vulgaris*, after light soil surface cultivation around 2 cm soil depth, application of linuron, linuron + thiobencarb, or S-metholachlor + thiobencarb before sowing or transplanting could be recommended.

## **Purple witchweed (*Striga hermonthica* (Delile) Benth.) *in vitro* germination and visualization of its cytoskeleton**

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Purple witchweed (*Striga hermonthica* (Delile) Benth.), Orobanchaceae) is an obligate root parasitic plant agronomically devastating to crop yields in the developing world causing enormous annual losses of finger millet, maize, sorghum, and upland rice. No effective control measures have been used against purple witchweed except phytoquarantine, *Striga*-tolerant cultivars and suicidal germination. In this study, high-throughput seed tetrazolium salt (MTT) germination bioassay for root parasitic plants with spectrophotometric reading of MTT reduction to formazan after the germination of *Striga* seeds in standard 96-well plate was used. This approach is widely employed in the testing of biological activity of commercial and newly synthesized strigolactones. The objective of this study was to optimize *in vitro* the protocol for *S. hermonthica* seed germination and examine the organization of microtubules and actin filaments in germ-tubes and prehaustorium tip cells of 7 days-old *S. hermonthica* seedlings using confocal laser scanning microscopy, whole mount immunolabeling of tubulin with the primary monoclonal anti- $\alpha$ -tubulin rat and the secondary anti-rat Alexa Fluor 488 antibodies as well as actin staining with phalloidin-Atto 488. It was found that the most efficient stimulant for the germination of *S. hermonthica* was exogenously applied synthetic strigolactone GR24 in concentration of  $3 \cdot 10^{-6}$  M that mimics the host root exudate. Other tested compounds were new hybrid type strigolactone-auxin mimics, some of which showed activity as high as GR24. Longitudinally oriented cortical microtubules, individual actin filaments and long cables of F-actin were present in actively elongating germ-tubes and prehaustorium tip cells of 7 days-old *S. hermonthica* seedlings. To our knowledge, this is the first report about the organization of cytoskeleton in root parasitic plants, which input to the understanding of their parasitizing strategies and can be used to develop the efficient protective approaches against highly invasive parasitic weeds.

## The effects of stratification and seed burial on Common ragweed seed germination and viability

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Common ragweed (*Ambrosia artemisiifolia* L) is a frequent weed species and also a major allergenic plant which causes severe health problems in humans. Plants produce large quantity of seeds which remain viable in the soil seed bank for decades. The objectives of this study were to determine the effects of various stratification regimes and seed burial on germination and viability of common ragweed seeds. Seeds were collected in autumn 2015 and analysed for initial germination and viability with Tetrazolium (TTC) test. A subsamples of 100 seeds were placed in mesh bags and buried into meadow at two depths (5 and 25 cm) while other seeds were subjected to different stratification regimes. The stratification treatments consisted of 3, 8 and 20 weeks of cold storage at 4° C with combination of short time freezing at -20° C. Although initial germination rate before stratification and seed burial was extremely low (5 %), however when stained with Tetrazolium (TTC) test, almost all fresh seeds were viable (98 %). When seeds were stored at 4° C for 2 months, germination rate increased to 70 %, while total viability remained very high (98 %). There were statistical differences in germination rate among different stratification regimes and seed burial (\*\*P<0.001). The highest germination rate (99 %) was observed when seeds were buried under ground over winter for 5 months, where all viable seeds germinated regardless of burial depth. Germination rate (87 %) of 5 months cold stored (4° C) ragweed seeds was significantly higher (\*P<0.05) compared to other treatments, while viability rate (97 %) was similar between all treatments. Short time freezing (-20° C) did not improve germination or total viability rate. Results showed that dormancy plays an important role in ragweed population dynamics and represent important factor in management plans of infested areas.

## Effect of constant and alternate temperatures on the germination of *Chloris elata* from Angola

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Germination represents an important passage for perennials since only seeds ensure the mid-long distance dispersion for these species. Improving the knowledge of germination process facilitates the design of rationale management strategies for perennials also because chemical or mechanical control tools can have a different efficacy on seedlings than on ramets. An experiment was conducted to evaluate the effect of temperature on the germination of *Chloris elata* collected in the area of Sumbe (11°11'42''S, 13°50'51''E, Kwanza sul, Angola). Seeds were incubated in Petri dishes at a range of constant (10, 15, 20, 25 and 30 °C) and alternate (15-5, 20-10, 25-15, 30-20 and 35-25 °C) with a 12 h light/12 h dark photoperiod. Germination was monitored daily. Three 50-seed replicates were included for treatment and the experiment was repeated twice. Data of the two experiments were pooled and mean plus standard errors were calculated for each treatment.

No germination was observed at 10 nor 15-5 °C, while germination percentage ranged from 32.7±2.81 to 39.3±3.82 % and from 26.3±0.95 to 39.7±2.85 % for the other alternate and constant temperatures respectively. The maximum germination percentages were obtained at 25-15 and 25 °C. First germinated seeds were observed at 2-7 Day After Sowing (DAS) at all temperatures and germination lasted until 15-46 DAS. Similar germination percentages were obtained with alternate and constant temperatures, while germination speed was lower, and consequently germination duration longer, at constant temperatures.

The fast and high germination of *C. elata* across a range of constant and alternate temperature indicates the ability of this species to produce relevant and concentrate seedling emergence flushes when soil conditions allow germination, causing potentially strong competition against the crop. This behaviour facilitates the control of *C. elata* early in the cropping season with stale seedbed techniques or pre-emergence herbicide application.

## Effect of constant and alternate temperatures on the germination of *Cyperus rotundus* from Angola

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The study of germination process of perennial weeds can improve the knowledge of their spatial dispersion ability and facilitate the design of sustainable management strategies, different control tools can indeed be adopted during the seed germination – seedling emergence phase.

An experiment was conducted to evaluate seed germination of *Cyperus rotundus* collected in the area of Sumbe (11°11'42"S, 13°50'51"E, Kwanza sul, Angola) at constant and alternate temperatures. Seeds were incubated in Petri dishes at a range of constant (10, 15, 20, 25 and 30 °C) and alternate (15-5, 20-10, 25-15, 30-20 and 35-25 °C) with a 12 h light/12 h dark photoperiod. Three 50-seed replicates were included for treatment and the experiment was repeated twice. Germination was monitored daily. Data of the two experiments were pooled and mean plus standard errors were calculated for each treatment.

Almost no germination was observed at all constant temperatures, while germination percentage was 0 at 15-5 °C and varied from 24.0±4.44 % at 20-10 °C to 38.3±3.59 % at 25-15 °C. First germinated seeds were observed at 11-34 Day After Sowing (DAS) and germination lasted until 70-130 DAS at 35-25 and 20-10 °C respectively.

The slow and prolonged germination of *C. rotundus* could reduce its competitive ability, since seedling emergence occurs progressively during cropping season, but also make more difficult to achieve optimal control. Herbicide application or mechanical operations should be postponed or repeated to eliminate all emerged seedlings. Since *C. rotundus* requires alternate temperature for germination, only seeds situated close to soil surface, where daily thermal fluctuations are maximum, can germinate. Seed burial by soil cultivation or the presence of living or dead mulches on soil surface can strongly inhibit germination of *C. rotundus* because the amplitude of thermal fluctuation to which seeds are exposed is limited under these conditions.

## **Translocation of macromolecules from tomato host plant to the parasitic weed *Phelipanche aegyptiaca***

Sally Marzouk, Radi Aly

Newe Yaar Research Center, RAMAT YISHAY, Israel

Broomrapes are holoparasitic weeds that lacking photosynthetic system and subsist on the roots of a variety of agricultural crops causing severe damage to numerous crops, reducing both yield and quality. In this study, we focused on *Phelipanche aegyptiaca* because it is serious pests in vegetable and field crops worldwide.

Broomrapes are able to connect directly with the vascular system of the host and thus uptakes water, minerals, and carbohydrates necessary for their own growth and reproduction. Until recently, little is known about translocation of mRNA from host plants to broomrapes. Therefore, we tried here to investigate this interaction.

In order to examine the translocation of mRNA from host plants to broomrapes, we selected five known genes that encode different proteins, identified in tomatoes and were shown to move from host plants to *Cuscuta* through the phloem tubes.

Tomato host plants were infested with *Phelipanche aegyptiaca* and translocation of mRNA to the parasite was tested after 7 weeks using RT-PCR and specific primers. According to our results, mRNA of the candidate five selected genes was able to translocate from host to the parasite tubercles.

In this study, translocation of Green fluorescent protein- (GFP) from host to parasite was also investigated. For this purpose transgenic tomatoes expressing GFP were inoculated with *Phelipanche aegyptiaca* and translocation of the protein was examined 4 weeks later. Our results demonstrate that GFP was able to move from the host roots to the attached parasite tubercles and shoots through the phloem system.

Study the translocation of macromolecules between parasitic weeds and their hosts is an important issue in regards of developing a new strategy for host resistance against the parasite.

## Genetic variability of *Echinochloa* spp. from Italian rice fields

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*Echinochloa* spp. have evolved resistance to both ALS- and ACCase-inhibiting herbicides, the most used herbicide sites of action in European rice fields. *Echinochloa* species are often difficult to distinguish due to the high morphological variability.

Objective of this study is to use DNA barcoding and species-specific (SS) PCR techniques to identify the most widespread species of *Echinochloa* spp. in Italian rice paddies.

Seeds of 20 morphologically different *Echinochloa* spp. plants were collected in 6 Italian rice fields. After phenotypic discrimination, made according to Costea-Tardif (2002) classification, gDNA was extracted and five regions of the cpDNA (3 genes: *matK*, *rbcl* and *trnF* and 2 introns: *trnL* and *psbA-trnH*) were amplified by PCR. Sequences were compared to already published sequences of *Echinochloa* spp. to check specie-specific mutations and phylogenetic trees were built. Subsequently specie-specific primers on SNPs identified on *matK* sequences plus one generic reverse primers were built and a protocol for SS-PCR was set up.

All of the five regions taken into account allowed discriminating *Echinochloa crus-galli* (also called »red species«) from the other species (also called »white species«). However, only *matK* gene and *psbA-trnH* intron led to a more precise distinction of the different species included into the *Echinochloa* »white species« group. Comparing *psbA-trnH* and *matK*, the latter was the most accurate as it clearly discriminated four groups: one with *E. crus-galli* samples only, two including »white« species of *Echinochloa* spp. and one that included sequences from four plants that had intermediate characteristics. The SS-PCR protocol discriminated three different species of »white« *Echinochloa* in one PCR reaction.

This type of analyses allows a high number of samples to be analyzed and classified in a short time contributing to a better knowledge of the different species of *Echinochloa* spp. infesting Italian rice and to devise more effective control strategies.



## Phenological development of *Echinochloa crus-galli* (L.) P. Beauv. populations at different geographical locations

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*Echinochloa crus-galli* is a highly competitive annual weed distributed across a wide climatic gradient. Appropriate timing is important for successful control, therefore modelling of phenological developmental stages can be a useful tool for a decision support system. Predicting flowering time is also useful to reduce seed production. Phenological development of *E. crus-galli* plants was studied in a common experiment by the EWRS working group Germination and Early Growth. Sowing was done in October–November 2015 and plant development was studied in the following vegetation season. Seeds from two common populations (Italy (South) and Norway (North)) and 1–3 local populations were sown in 25 cm pots at each of the study sites to monitor emergence. In each pot three plants were preserved for monitoring development using BBCH scale. Soil disturbance and fertilization was performed in March–April 2016. Time required for the plants of different populations to reach certain development stages at different sites was analysed. There was a significant difference in the time required to reach BBCH12 (two leaves unfolded) between the locations, but no differences between the populations, except in Poland. Location was a significant factor for time to reaching BBCH23–25 (3–5 tillers), but no difference was detected among populations except in the location in Italy. However, timing of the onset of flowering (BBCH over 49) was different and was significantly delayed in plants of South population at several locations, while plants of the North population developed faster. This can be explained by quantitative photoperiodicity in *E. crus-galli* (a short-day plant) that was pronounced in the South population but not in more northern populations. The data will be further used to develop models of early development of *E. crus-galli*. The possibility of using the cumulative degree-days to model its phenological development is discussed.

## Evaluation of light/temperature on the germination of *Conyza canadensis* (L.) Cronq. in central Italy

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Global climate change and invasive species are quickly becoming two of the most important problems in natural and human managed ecosystems. *Conyza canadensis* (L.) Cronq is one of the most invasive plants in the world, widespread in the European and Italian agricultural systems. Temperature and light have been known to influence weed seed germination, but little information exists about the effect of environmental factors on *C. canadensis* germination. The objective of this study was to determine the effect of temperature and light on *C. canadensis* germination. Seeds of *C. canadensis* were collected from naturally senescing plants in central Italy (September 2016) and stored in the dark at 4 °C. Fifty seeds were placed on a filter paper (moistened with 5 ml of deionized water) in 90-mm plastic Petri dishes that were put in a growth chamber at different light/temperature conditions: eight constant temperatures (2.5, 5, 10, 15, 20, 25, 30, 40 °C) and three fluctuating temperatures (18/12, 23/17, 28/22 °C) in 12h/12h:light/dark; three fixed temperatures (15, 20, 25 °C) in complete darkness. All treatments were replicates thrice. Seed germinations were recorded daily until no more germinations were observed for at least 7 days. *C. canadensis* was able to germinate in a wide range of temperatures ( $T_{\text{base}} = 6.7$  and  $T_{\text{cutoff}} = 35.6$  °C). The highest germination capacity was observed at 15 °C (light/dark); no differences were observed at 17/23 and 22/28 °C with respect to 20 and 25 °C (light/dark), while germinations were significantly reduced at 12/18 °C and in darkness. The highest germination rate was observed at 25 °C (light/dark) and it was significantly reduced at 12/18 °C and in darkness. These results can help understand the potential for *C. canadensis* to spread into new environments and agro-ecosystems, as well as suggest new methods and strategies for its management.

**Synergism in the germination stimulation of *Orobanche* and its effect on host specificity**

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Obligatory parasitic plants of the Orobanchaceae are among the most persistent and economically damaging agricultural weeds, posing major constraints to the production of many crops in the Mediterranean, Asian, European and African regions. Their seeds germinate only in response to chemical stimulants, which are released by potential host plants. Many studies focused on the identification of stimulants from various host plants, and it is currently widely known that the main group of stimulants is plant hormones belonging to the strigolactone (SL) family. Further research has shown that plants exude mixtures of various SLs, as well as other compounds that may serve as germination stimulants for some parasite species. In all these cases, seed germination was shown to be related to stimulant concentration. We hypothesized that parasitic plants may select their hosts by specifically responding to stimulant combinations rather than to a single stimulant. We further hypothesized the occurrence of synergistic effects of stimulant combinations on the germination response. To this end we examined the response of *O. cumana* - a specific parasite of sunflower, and of *O. cernua* - a parasite of tomato, to strigolactones and sesquiterpene lactones (STLs) that are exuded by their respective hosts, and examined the stimulation effect of combinations of various concentrations of these stimulants. A clear synergistic effect was found between SLs and the STL dehydrocostus lactone, which is consistent with the host preference of the two parasite species. We therefore conclude that the germination of obligatory parasitic Orobanchaceae specifically requires the presence of a certain stimulant combinations in the host rhizosphere rather than the presence of a certain single stimulant.

## Characterization of a diverse hybrid population of *Phelipanche ramosa* and *P. aegyptiaca*

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*Phelipanche aegyptiaca* and *P. ramosa* are closely related, but have often been confused. Here we report on a highly diverse *Phelipanche* population that was found in a tomato growing region in Israel. This population reflects the dynamic nature of the two parasitic weeds when they happen to grow together. Parasite specimens were collected in this field in August 2015 and characterized for their height, flower density, flower length, and stamen hairiness. Using these diagnostic characteristics the specimens were found to belong to three main groups: (a) the *Ramosa* Group, (b) the *Aegyptiaca* Group and (c) the much larger group of specimens exhibiting a wide range of intermediates, varying separately in each of the examined characteristics. Interestingly, the specimens collected in the same place in 1987 were rather uniform in all these characteristics, and easily identified as *Phelipanche ramosa*. Tomato is commonly grown in this region only for the last two decades, and potato was the former dominant crop. Whereas potato was typically infested with *P. ramosa*, *P. aegyptiaca* is typically infesting tomato fields in Israel. It seems that *P. aegyptiaca* seeds were transferred to the region by the communal harvesting equipment, which serves infested and non-infested areas. The research results demonstrate that when seeds of two parasitic weeds are transferred to the same field, they may potentially form a diversity of hybrids differing in their morphological characteristics. The evolution of such hybrids may potentially lead to the evolution of more virulent parasitic weeds and extend their host range.

### **Emergence of *Avena sterilis*, *Centaurea diluta*, *Chrysanthemum segetum*, *Lolium rigidum* and *Ridolfia segetum***

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*Avena sterilis*, *Centaurea diluta*, *Chrysanthemum segetum*, *Lolium rigidum* and *Ridolfia segetum* are problematic weeds in different regions of Spain. For this reason, the Biology and Agroecology Group of the Spanish Weed Science Society set an experiment to study their emergence and growth in 11 different sites distributed throughout Spain. Seeds of one population per species were collected in June 2016, and in each site 100 seeds of each species were sown in October-November 2016, disturbing the soil down to 2 cm (10 cm for *A. sterilis*) in planting marks divided in six 0.25 × 0.25 m<sup>2</sup> cells, with four replications. Samplings were performed every 2-7 days. In the present work the emergence results are presented and the results show that *C. diluta* and *L. rigidum*, followed by *A. sterilis*, were the first emerging species. These three species were also those which showed higher emergence percentages. The emergence timing, counted as number of days from sowing, varied also between sites for each species, although *C. diluta* and *L. rigidum* seem to have lower dormancy levels and these differences between sites are diminished. *A. sterilis* and *R. segetum* appeared to be more affected by the site factor, which could be explained by higher dormancy breaking requirements, which could have varied between sites. Finally, *C. segetum* showed very low emergence, below 10% in most sites, which prevents to develop in this species the emergency models that are going to be implemented in the other ones.

### **Effect of salinity on germination and growth of *Echinochloa crus-galli* and *Oryza sativa***

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Salinization is a phenomenon of growing importance in several regions, including European rice growing areas. Salinization may directly affect rice, but it can also have an influence on weeds: among other effects, it could be hypothesized that salinity may interact with herbicide resistance. The study was aimed at evaluating the effect of salinity and herbicide application on germination and growth of barnyardgrass (*Echinochloa crus-galli*) (both sensitive and resistant to ALS-inhibitors) and weedy rice (*Oryza sativa*) populations. Trials were conducted in greenhouse; plants were grown in alveolar trays filled with sand and placed in basins containing growing solutions with salt concentrations ranging from 0 mM NaCl to 250 mM NaCl. The results showed a reduction of germination capacity, speed of germination, plant height, shoot and root fresh weight with increasing saline concentrations. A variable salt response was observed for SPAD (soil plant analysis development) readings on leaves, which are correlated to chlorophyll activity, and content of chlorophyll and carotenoids. Barnyardgrass populations showed a moderate salt tolerance for concentrations up to 150 mM NaCl with an average plant height, shoot and root fresh weight of 23.52 cm, 2.02 g and 3.04 g, respectively. The resistant population exhibited a salinity tolerance greater than that of the sensitive ones. Tolerance to salinity in weedy rice was lower than that found for barnyardgrass: seedling emergence occurred at concentrations up to 100 mM but it was followed by plant desiccation in few days. Response to herbicide application was only poorly influenced by salinity; in general, herbicide-treated plants showed a decrease in plant height, chlorophyll and carotenoids content, when grown under saline conditions. The good ability to adapt to the salt conditions shown by the barnyardgrass suggests that this weed would still to be highly problematic in rice field even in a scenario of increased water salinity.

## **Seedling Emergence and development of *Chenopodium album* affected by Soil Disturbance and Crop Competition**

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To study the effect of soil disturbance on seedling emergence and development of *Chenopodium album* a pot experiment was carried out outdoors during 2017. In spring 100 seeds/pot of *C. album* were sown at the soil surface with three soil disturbance treatments -no disturbance, disturbed down to 5 cm (0-5) and disturbed down to 15 cm (0-15)- and with and without red bean (*Phaseolus vulgaris* L.). Four replicates were installed for each treatment. The bean seeds were sown at 5 cm depth. The number of emerged seedlings of *C. album* was recorded every 3 days. Three seedlings of *C. album* and bean that emerged simultaneously were chosen for recording the phenological development using BBCH scale. Result showed that the emergence of *C. album* was significantly affected by type of soil disturbance, but not by the presence of the crop. The number of emerged seedlings was higher at soil surface than in the soil disturbed treatments, and lower at 0-15 cm soil disturbed treatment. Phenological development of *C. album* was similar in all treatments until BBCH 11, after which the development of *C. album* became faster for plants grown without the crop. For *C. album* plants grown in competition with bean, phenological development was faster after BBCH 12 for plants that emerged from 0-15 cm than those from 0-5cm, and it was slower for plants emerged at the soil surface. It is concluded that both soil disturbance and crop competition affect the number of emergences and the phenological development of *C. album*, and this knowledge can be considered for the management of this weed species.

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## **Emergence and growth of *Cynodon dactylon* at two tillage timings in a semi-arid environment**

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The ability of bermudagrass (*Cynodon dactylon* (L.) Pers.) to spread through tillage and the lack of effective herbicide control make of it a noxious weed species in vineyard. With the aim of knowing the emergence and spreading capacity of this grass, a field experiment was conducted during 2016/17 season in Lleida (Cataluña, Spain). Rhizome and stolon fragments were collected in a vineyard and classified in three different sizes, one, three and six nodes. Two rhizomes or stolons were buried in 20 cm diameter pots at 1, 5 and 10 cm, with five replications. The experiment was set in November 2016 and repeated in January 2017, to simulate two disturbance dates. Pots were not irrigated, and emergence was monitored daily until summer, and weekly during summer. Pots were removed in late September and the following variables were measured: size of the main rhizome and stolon, number of nodes, shoots and panicles, and above and below ground dry matter was weighted. Results show that tillage timing is the main factor that affects the emergence and the establishment of the weed: up to 58.9% of the individuals died when the experiment was established in November, when rhizomes and stolons were still active, while this mortality was reduced to 26.7% when it was set in January, when they were dormant. One node organs also survived in less proportion than three-node and six-node organs. Burial depth was important for one-node organs, while three-node and six-node organs were less affected by this factor. Rhizomes tend to survive in a greater quantity than stolons when buried. These results suggest that the best non-chemical control method would be an intense tillage in autumn, but it must be confirmed with a repetition of the experiment in a second season.



**S10-O Black-Grass  
Resistance Initiative  
ORAL PRESENTATIONS**

## Characterisation of a glutathione transferase dependent pathway in multiple herbicides resistance in *Alopecurus myosuroides*

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Non-target-site based resistance is a polygenic trait associated with the upregulation of proteins involved in herbicide detoxification and translocation called the xenome. Among these proteins glutathione transferases (GST) can achieve enhanced herbicide metabolism by conjugating GSH to xenobiotics and ABC transporters can increase the rate of herbicide translocation into the vacuole. Based on proteomic and transcriptomics data we previously identified in Black grass (*Alopecurus myosuroides*, *Am*) a GST from the Tau subfamily (*AmGstu2*) that is upregulated in multiple herbicides resistance (MHR) populations but not under other stress conditions, making it a good marker for herbicide resistance. GSTs are a family of enzymes that carry out a wide range of enzymatic and non-enzymatic functions. A phylogenetic analysis revealed that *AmGstu2* clades together with *TaGstu4*, a protein from wheat with GST activity towards herbicides. Consistent with this, *AmGstu2* is able to conjugate Gluthatione (GSH) to xenobiotics and the mutation of its catalytic residue (S12A) abolished this activity. This contrasts with the activity of *AmGstf1*, a protein also associated with MHR populations but that lacks GST activity towards xenobiotics, suggesting that proteins from the same family can contribute to the resistance phenotype through different mechanisms. We analysed the transcript profile of *AmGSTU2* through populations of Black grass with different degree of resistance to herbicides and compared it with the expression of two ABC-C transporters and *AmGSTF1*. We found that the expression of *AmGSTU2* strongly correlates with the two ABC transporters and the three genes are mainly up regulated in populations with high levels of herbicide detoxification rates. These results support a model in which *AmGstu2* and the two ABC transporters, but not *AmGstf1*, are part of the same stress response pathway and their contribution to the resistance phenotype is probably directly linked to herbicide metabolism.

**On-Farm diagnostics for herbicide resistance testing in *Alopecurus myosuroides***Nawaporn OnkokesungSchool of Natural and Environmental Sciences, Newcastle University, NEWCASTLE UPON TYNE,  
United Kingdom

Populations of black-grass (*Alopecurus myosuroides*) that are resistant to multiple classes of herbicides are now a prominent problem that challenge cereal crop productions in Northern Europe. As part of a UK national black-grass resistance initiative (BGRI), our group at Newcastle University are interested in the mechanisms underpinning non-target site resistance (NTSR) to herbicides in black-grass. Using a combination of transcriptomics and proteomics, we have identified a glutathione transferase phi-class 1 (AmGSTF1) protein that is functionally linked to non-target site resistance (NTSR) to herbicides in black-grass populations isolated from the fields in the United Kingdom. Furthermore, we have also demonstrated that overexpressing AmGSTF1 in *Arabidopsis thaliana*, conferred resistances to multiple herbicides in transgenic plants.

Based on the role of AmGSTF1 as a functional biomarker of NTSR, has allowed us to develop a low-cost in-field diagnostic using lateral flow device (LFD) technology that gives a quantitative read out of AmGSTF1 protein content in black-grass plants within 10 minutes. By screening multiple black-grass populations varying in their resistance to herbicides, we have established a positive correlation between the LFD read out, AmGSTF1 protein abundance and the levels of NTSR exhibited. Currently we are working on translating this technology into robust applications for use in the field that will provide real-time information for monitoring and mitigating herbicide resistance in black-grass populations in the hands of agronomists and growers. Future work is focussed on developing second generations of diagnostics for detecting multiple biomarker proteins and genes for both NTSR and Target Site Resistance in black-grass and other grass weeds.

## The evolutionary dynamics of NSTR in *Alopecurus myosuroides*

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*Alopecurus myosuroides* with evolved resistance to multiple herbicide modes of action is widespread in winter wheat fields in the UK. There are two main categories of herbicide resistance in *A. myosuroides*; target-site resistance (TSR) and non-target-site resistance (NSTR). Although TSR is well-characterised, questions remain about the biochemical and genetic mechanisms that underpin NSTR, however enhanced metabolism is known to play a key role. Importantly, we also have little understanding of the inter- and intra-population variability in NSTR, nor the mechanistic basis for different patterns of cross resistance. Here, we investigate variation in the NSTR phenotype and genotype amongst 27 populations of *A. myosuroides* collected from winter wheat fields in the UK. The resistance phenotype of these populations to fenoxaprop-P-ethyl, cycloxydim and mesosulfuron/iodosulfuron was established through glasshouse dose response experiments, and a variety of cross-resistance phenotypes were identified. In order to determine the contribution of herbicide metabolism (NSTR) to resistance phenotypes, we applied radio-labelled herbicides (mesosulfuron and fenoxaprop-P-ethyl) to excised leaf tissue from growing seedlings, and used HPLC analysis to identify herbicide metabolites. We also measured the relative expression of a set of putative NSTR biomarkers in these populations to find the underlying patterns of gene expression which correspond with these metabolic resistance phenotypes. Two GSTs and a CYP450 had low expression levels in more susceptible populations and higher (but variable) expression levels in populations with higher levels of resistance. We have combined these whole plant and metabolism phenotypes with transcript expression data using multivariate statistical analyses to better understand how different herbicide metabolism phenotypes correspond to the various patterns of transcript expression observed in these populations. Using this approach, we explore patterns of among-population variation in NSTR and begin to establish how different cross-resistance phenotypes are underpinned by the differential expression of a suite of diagnostic expression-based markers.

## **The epidemiology of herbicide resistance evolution in *Alopecurus myosuroides***

David Comont

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*Alopecurus myosuroides* is the predominant weed species affecting arable cropping in the UK, with its impact exacerbated by evolved resistance to multiple herbicides. Controlling this species is further complicated by the co-existence of multiple resistance mechanisms, including both specific (TSR) and non-specific (NTSR) resistance. Here, we present a large-scale epidemiological analysis to investigate the evolution of herbicide resistance in *A. myosuroides*. We have investigated the extent and mechanistic basis of herbicide resistance in over 100 field collected populations of UK *A. myosuroides*, using a combination of dose-response phenotyping, target-site sequencing, and quantification of the AmGSTF1 protein biomarker for NTSR. Relationships between current weed population densities, herbicide resistance, and field management histories (herbicide applications, crop rotations and cultivation regimes) have also been assessed, to evaluate the key agronomic factors driving herbicide resistance evolution in this species. We find that herbicide resistance is widespread, with the majority of populations resistant to multiple herbicide modes of action. Mutations within the target site of the acetyl CoA carboxylase (ACCase) and acetolactate synthase (ALS) genes are frequent, while non-target-site resistance is also found to be widespread, often co-occurring with TSR. Weed densities are correlated with the presence of evolved resistance, suggesting that resistance is facilitating increased weed abundance at a national scale. The frequency of herbicide applications proved to be the strongest predictor of current herbicide resistance, while other cultural techniques were found to have limited impact, confirming that the intensity of herbicide exposure provides the principal selection for resistance. Specific associations between management, mechanism and phenotypic resistance to individual modes of action are further explored to detect more subtle signatures of selection. These results highlight the value of a large-scale epidemiological approach to understand the evolution of pesticide resistance.

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**Calculating the cost of herbicide resistance in *Alopecurus myosuroides* (Blackgrass).**

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Selective pressure due to overuse of herbicides is causing resistance to these chemicals, compromising agricultural productivity. In an era of increasing human population and concerns about food security, herbicide resistance is a pressing global issue. The economic costs of herbicide resistance are thought to be very large; however, country-scale estimates of these costs are absent, despite their importance in helping decision-makers assess the relative costs of pre-emptive mitigation vs inaction. We used national scale density data for the weed *Alopecurus myosuroides* (Blackgrass), combined with crop yield maps and a new economic model, to estimate regional and country-level costs of resistance in English cereal farming. Mean cost per hectare, in terms of management costs and lost yield, ranged from £90/ha at low Blackgrass density to £450/ha at very high Blackgrass density. These scale up to huge costs: in the East of England, for example, Blackgrass is costing farmers £126 million per year. With 253 herbicide-resistant weeds worldwide, the global costs of resistance will run into the billions. The model is now being used to assess the economic outcomes of different management strategies aimed at mitigating herbicide-resistant Blackgrass. In addition, environmental models will assess these same strategies to estimate various environmental outcomes, enabling an analysis of financial and environmental synergies and trade-offs for each strategy.

**S11-O Integrated Weed  
Management  
ORAL PRESENTATIONS**

## Winter cover crops to reduce herbicide use in New Zealand maize crops

Trevor James

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In New Zealand, maize (*Zea mays*) is often grown as a monoculture with a winter fallow. Our objective was to evaluate the feasibility of winter cover crops to improve weed control and reduce herbicide inputs in maize cropping.

Five winter cover treatments; gland clover (*Trifolium glanduliferum* cv. Prima), faba bean (*Vicia faba* cv. Ben), oats (*Avena sativa* cv. Milton), ryegrass (*Lolium multiflorum* cv. Tama) and fallow, were planted in autumn as main plots and replicated four times. These crops were treated with glyphosate (1458 g ai/ha), except faba bean (paraquat, 800 g ai/ha), 3 days prior to spring maize planting. Associated with the maize planting five herbicide treatments, no herbicide, acetochlor + saflufenacil (2520 + 105g ai/ha) pre-emergence, acetochlor + saflufenacil (2520 + 105g ai/ha) pre-emergence followed by topramezone and atrazine (67 + 500 g ai/ha) post-emergence, topramezone and atrazine (67 + 500 g ai/ha), mesotrione and atrazine (96 + 500 g ai/ha) early followed by nicosulfuron (60 g ai/ha) late post-emergence, were applied as randomised as subplots. Herbicides applied with a CO<sub>2</sub> powered backpack sprayer with a 3 m boom with four TeeJet 11003 AI nozzles at 160 kPa to apply 200 L/ha. Weed growth and maize production were measured.

The herbicides provided 98-100% weed control. In the no-herbicide plots weed cover was; clover 8%, oats 8%, ryegrass 10%, faba beans 22% and fallow 51% (LSD=7%). Maize silage yields (pooled over all subplots) were; clover 24.7 t/ha, oats 19.2 t/ha, ryegrass 17.6 t/ha, faba beans 23.2 t/ha and fallow 21.5 t/ha (LSD=3.3) and maize grain yields were; clover 12.5 t/ha, oats 9.4 t/ha, ryegrass 8.3 t/ha, faba beans 12.8 t/ha and fallow 11.2 t/ha (LSD=1.4).

Winter cover crops such as gland clover can reduce the need for prophylactic use of herbicides to manage weeds, without compromising maize production.



## **Reduced herbicide use does not increase crop yield loss if compensated by alternative measures**

Nathalie Colbach, Stéphane Cordeau  
INRA, DIJON, France

Herbicide use must be reduced because of environmental and health issues. To evaluate whether weeds and crop yield loss will increase, we collected data on 272 arable cropping systems from Spain and France, from farm surveys, the Biovigilance-Flore network, expert opinion, cropping-system trials, crop advisors and scientists. Each system was simulated over 27 years and 10 weather repetitions, using FlorSys. This process-based model simulates multispecies weed floras and crop canopies in virtual fields from cropping systems and pedoclimate at a daily time-step. Four simulation series were run, 1) starting with a typical regional weed flora, 2) eliminating all herbicides without any other change in management practices. The two series were run again, this time without weeds (series 3 and 4). Comparing series 1 and 2 to respectively 3 and 4 gave the crop yield loss due to weeds in series 1 and 2. Comparing series 1 to 2 quantified the herbicide impact on weeds and crop production. The simulations showed that (1) yield loss increased with increasing weed biomass, and that the weed/crop biomass ratio at crop flowering was the best indicator of the year's yield loss, (2) herbicide use intensity (TFI) was not correlated to either weed infestation or yield loss, because it greatly depended on other management practices, (3) weed biomass and yield loss increased when herbicides were eliminated without any other change in management practices, (4) effects were more visible at the multiannual than the annual scales. Monotonous herbicide-intensive rotations with short crop cover, no plough or winter ploughing and frequent rolling operations were the most sensitive to herbicide suppression. A decision tree predicting yield loss from management practices was built to support farmers and crop advisors when designing cropping systems reconciling low herbicide use and low yield loss.

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## **Egyptian broomrape (*Phelipanche aegyptiaca*) control in processing tomato: a review**

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Broomrapes (*Orobanche* and *Phelipanche* spp.) are root parasitic plants that cause severe damage and great yield and quality losses in vegetables and field crops in Mediterranean, Eastern and Southern Europe. In Israel, Egyptian broomrape (*Phelipanche aegyptiaca*) is a most devastating pest, parasitizing a wide host range including tomato, sunflower, legumes and carrot, resulting in severe economic losses. Broomrape control is complicated because it's unique biological traits that are highly specialized for parasitism, and that most of the damage caused by the parasite occurs before its above ground emergence. Two decades of studies in Israel led to development of integrated smart management strategies for efficient control of Egyptian broomrape in processing tomato production. These studies resulted in several options for control that were integrated into a decision support system (DSS) named '*PICKIT*'. This DSS predicts parasitism dynamics for temporal precision chemical control with different application methodologies, including pre-planting incorporation, foliar application and application of herbicides via irrigation. In this review we describe the research progress from the lab, through greenhouse and experimental field trials to large scale commercial fields and its successful implementation in Israeli agricultural. The success of the DSS increases tomato yield and quality in all Egyptian broomrape infestation levels and in high field infestation levels tomato yields increase by an average of 40 tons ha<sup>-1</sup> compared to non-treated fields. DSS *PICKIT* was commercially used in 2016 and in 2017 in 74 fields totaling 1100 ha, resulting in 95% Egyptian broomrape control and high tomato yields of 115-145 tons ha<sup>-1</sup>. The implementation *PICKIT* enables farmers to grow tomatoes on Egyptian broomrape-infested fields, assuring increased yields and high profits.

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**Knowing your enemy - a route to optimum weed population management**Lammert Bastiaans

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The sustainability of herbicidal control is at stake, as herbicide resistance and stricter regulations towards admission of herbicides have reduced the number of available compounds. Consequently, farmers depend on a smaller number of products and this increases the likelihood of herbicide resistance to develop, undermining not only the effectiveness, but also the sustainability of weed control. Alternative curative control measures combining a high efficacy with a relatively low time investment are scarce. The implication is that curative control will have to be supplemented with other measures, like cultural control. Cultural control measures are not restricted to the weed seedling stage, but can be targeted at any life cycle stage. The aim of the current study was therefore to understand how various life cycle stages interact in governing the size of the weed population and to unveil weak spots in the weed's life cycle. For this study a simple weed population model was used to analyse the secrets behind the life cycle dynamics of weed populations. The model consisted of a coherent set of life cycle stages and the transition rates between them. All transition rates combined determine the equilibrium weed density, which was used as a proxy for the potential threat of the weed. Sensitivity analysis was used to identify vulnerable stages of the weed. The framework was also used to study how the success of a cultural control measure depends on the life cycle traits of a weed species and how and to what extent different measures interact. Finally the relation between the level of curative weed control and the effectiveness of cultural control measures was investigated. Such insights are instrumental for developing novel integrated weed management strategies that aim to exploit cultural control options to its full potential.

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## **How tillage date, herbicide use and Harvest weed seed control affect weed emergence dates**

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Tillage, herbicides and Harvest Weed Seed Control are used in cropping systems to reduce both the number of weeds, and the size of the weed seedbank. However, the influence of the repetitive use of these management strategies on seedling emergence dates has not been investigated. A simple spreadsheet program (available on request) has been designed to illustrate how tillage date, herbicide use, and HWSC, will all influence selection pressure on the emergence date of weeds. Without herbicide use, or HWSC, weeds will germinate as early as possible, to maximise their competitive ability. In contrast early season tillage and herbicide use are shown to increase selection pressure for later weed seed germinations by a factor of 10, in annual ryegrass. However, the addition of HWSC promoted earlier season germination, and reduced to ¼ the increase in selection pressure for late season germination. Our results highlight the potential benefits of combining early season tillage and herbicide use with late season HWSC for both controlling weed populations, and reducing the future evolution of delayed weed emergence.

**S11-P Integrated Weed  
Management  
POSTER PRESENTATIONS**

### **Foliar fertilizer increases herbicide tolerance in maize inbred lines**

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Plant response to herbicides is very important in agriculture, especially in maize seed production. Due to inbreeding process, inbred lines are more susceptible to herbicides comparing to hybrids. Herbicide application is still the most effective method for weed control. Also, insufficient herbicide selectivity can be limited factor in seed crop production. Herbicides can cause visual plant damages or slow down plant development and finally decrease grain yield. On the other hand, dominance of grass weeds in maize crop occurs due to a lack of selective herbicides for their control. With sulfonylurea herbicides this problem became under control, but a problem with selectivity is still present, particularly in maize seed crop. Optimal plant nutrition provides better crop fitness and higher tolerance to herbicides. In case of foliar fertilizing, fast entry of macro- and micro-elements into plants also influence better plants response to herbicides and other negative impacts.

The effect of sulfonylureas and foliar fertilizer on five maize lines was evaluated in three year field experiment. This was done by visual estimation, grain yield measuring, as well as the alterations in the content of antioxidants such as phenolics free thiolic groups, and soluble proteins in the leaves. Positive effects of applied foliar fertilizer were observed on grain yield. Most of the genotypes expressed significant increase of grain yield in the treatments with foliar fertilizer, compared to control and analogous treatments with herbicides. Alternations in free thiolic groups and phenolics content was significantly influenced by applied treatments. The differences in the content of phenolics and thiolic groups in the treatments with herbicides + foliar fertiliser indicated that herbicide stress was more rapidly overcome. On the other hand, soluble protein content did not vary significantly maize leaves from observed treatments.

**Integrated Weed Control with 'DSS-IWM', an improved European Decision Support System**

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In the frame of the European ERA-Net project »Coordinated Integrated Pest Management in Europe (C-IPM)« scientists from Germany, Denmark and Spain design and customise an innovative online decision support system for integrated weed control (DSS-IWM) in maize and winter wheat. The project runs from 2016 to 2019 with the aim to assist farmers and farm advisors in treating weeds in crops at precisely the right times and the most efficient products in the right amounts. DSS-IWM can, therefore, contribute to reducing herbicide consumption markedly without affecting the yield. It will support reliable decisions based on local conditions and will consider thresholds for weed densities, include economic calculations of treatment costs.

The basis of herbicide recommendations is the database and the calculation/mathematics of the DSS-IWM, especially dose-response-relations of herbicides. If data gaps appear pot trials with respective weeds and herbicides are carried out. New features and information are continuously filled in. Additionally, in all countries field trials in maize and winter wheat are carried out to validate the DSS.

The main results of the new scheduled project will be the following: DSS-IWM is ready to online use for weed control in maize and winter wheat provides reliable decisions and considers national conditions enables to consider thresholds for weed densities includes economic calculation on treatment costs offers mechanical options wherever possible facilitates herbicide resistance management is the basic platform for uses in other crops and countries

The project is funded by the European Union in the Seventh Frame Work Programme, KBBE.2013.1.4-02: Integrated Pest Management (IPM) – ERANET Coordination and Support Action, Grant agreement no.: 618110

## How to eliminate obstacles of IWM implementation into cropping systems in South East Europe

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In this presentation, the outcomes of a specific EWRS regional meeting organized by the Working Group »Optimization of Herbicide Use in an IWM context« are documented. Data were obtained by a questionnaire survey and a face-to-face group discussion. The idiosyncratic structure and systemic nature of IWM systems, as compared with other IPM systems, draws on many strategies with a combination and integration of single weed management tactics at temporal and spatial scale. The questionnaire included queries with multiple-choice predefined answers and left space for open answers. All those were given a significance score value (1 to 5). During the meeting, the face-to-face group discussion was aimed on the fine elaboration, sorting and ranking of the major weed problems and obstacles for IWM systems implementation in South East European countries. Regional South East countries address the IWM by different approaches based on their status towards EU. Countries outside the EU, have set up voluntary public and private entities to promote IWM whereas Member States of the EU have implemented the Sustainable Use Directive (Dir. 2009/128/EC) aiming to regulate use of pesticides (herbicides, in particular), and are required to set up National Plans. Regional cropping systems frequently face similar (more or less) major weed problems and IWM challenges, research needs and priorities and extension services upgrades to tackle IWM implementation. Identifying the current obstacles and propose measures to eliminate them would boost national efforts as they could benefit from a common IWM framework and transnationally approaches.



**CA-SYS: A long term experimental platform on agroecology at various scales**

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The French National Institute for Agricultural Research (INRA) has established an ambitious, multi-scale, agricultural experimental infrastructure (the CA-SYS platform). CA-SYS covers an area of 120 ha and will be initiated in autumn 2018. The aims of CA-SYS are to: i) design and evaluate new agroecological systems; ii) study the transition from current farming systems towards these new agroecological systems, with goals that include agronomical performance, the evolution of farming practices and multi-performance criteria; iii) breed new varieties adapted to agroecological conditions, for example tolerance to stressors and the enhancement of beneficial plant-microbe interactions; iv) understand the ecological processes underlying the functioning of agroecological systems; and, v) develop and adapt experimental methods for studying agroecological systems.

The originality of CA-SYS is that it is explicitly conceived for the development and evaluation of new agroecological systems across agriculturally realistic scales. An agroecological system will comprise a matrix of fields of one (or a few) cropping systems over a number of years. These fields will interact with adjacent semi-natural habitats in the landscape (woods, hedges, grass margin strips, flower strips). This spatio-temporal arrangement of fields and semi-natural habitats will be considered as a coherent strategy, implemented to meet specific goals. The agroecological systems tested across CA-SYS will consist of three zone of manipulation of the amount of adjacent semi-natural habitats available to enhance the natural enemies of pests and four cropping systems combining a large diversity of farming practices (no-till & cover crop based-systems, tillage-based systems).

CA-SYS has ambitious objectives, including an increase in the multi-performance of systems (profitability and productivity identical to neighbouring farmers over a 10 year-horizon, low environmental impact), by maximising the use of biological processes (biological control of pests, improving nitrogen cycling) and reducing the use of inputs (nitrogen, water, pesticides).

## **Weed control in rice grown with plastic mulching and drip irrigation system**

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Plastic mulching and drip irrigation in organic rice cultivation are practices aimed at controlling weeds and saving water. A study was conducted in 2016 and 2017 to test the combination of both techniques in a rice field in N-W Italy. The field was equipped with a subsurface drip irrigation system and subdivided in three plots (split-plot design), in which rice was mulched with biodegradable films of different thickness: one black in color and with a thickness of 15  $\mu\text{m}$  and two with a thickness of 12  $\mu\text{m}$ , one black and one transparent. An adjacent drip irrigated field was dry seeded and used as a reference. Rice seeding was performed in May and the mulched plots were sown with a plastic sheet laying and planting machine. The mulched strips were 140 cm wide and hosted 5 rice rows, while the bare soil between the mulched strips (inter strip) was 70 cm wide. The amount of water supplied to the field over the whole growing season was about 4500  $\text{m}^3/\text{ha}$ . Weeds grown in the inter strips were controlled with a modified inter-row hoeing. During the season, weed and rice density, rice height, number of dead rice plants, weed control efficacy in the inter strips and rice yield were assessed in all plots. The result of the study showed that in the inter strips, weed density at the final assessment was higher in the 15  $\mu\text{m}$  black film (403 plants  $\text{m}^{-2}$ ) and lower in the transparent film (126 plants  $\text{m}^{-2}$ ), while the number of rice culm per meter had an opposite trend. Highest yield was recorded in conventional field (7.8  $\text{t ha}^{-1}$ ), followed by 15  $\mu\text{m}$  black film with 3.7  $\text{t ha}^{-1}$ , while the lowest were those recorded with 12  $\mu\text{m}$  black and transparent films (2.3 and 2.4  $\text{t ha}^{-1}$ , respectively).

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**The Use of Siam Weed (*Chromolaena odorata*) as Organic Mulch to Control in Soybeans**

Hasanuddin Hasanuddin, Hafsa Siti, Resdiar Amda  
Syiah Kuala University, BANDA ACEH, Indonesia

One of organic mulches that can be used to control weeds on soybeans plantation is siam weed. A research has been conducted to determine the right dosage and application time of organic mulch siam weed to control weeds in soybeans plantation. The research was held in April until June 2015 in Rumpeet Village, District of Krueng Barona Jaya, Aceh Besar, Indonesia. The research was assigned in Randomized Completely Block Design Factorial. The first factor used was dosages of mulch: 6, 12, and 18 ton ha<sup>-1</sup>, while the second factor was application time: on planting, one week after planting, and two weeks after planting. The variables observed were percentage of weed coverage, dry weight of weeds, crop growth rate, number of pods per plant, number of seeds per plant, weight of seeds per plant, and yield of dry seeds. The result showed that dosage of mulch affected the crop growth rate. Dosage of mulch at 18 ton ha<sup>-1</sup> could increased crop growth rate. Time of application affected the dry weight of weeds, number of pods per plant, number of seeds per plant, weight of seeds per plant, and yield of dry seed. Application time at on planting could decreased dry weight of weeds and increased number of pods per plant, number of seeds per plant, weight of seeds per plant, and yield of dry seed.

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## **Critical Time for Weed Removal in Soybeans as Influenced by PRE Herbicides**

Stevan Knezevic

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Increased POST applications of glyphosate based products for weed control in soybeans, caused rapid increase in glyphosate resistant weeds in USA. This led to an increased need to diversify weed control programs and use pre-emergent (PRE) herbicides with alternative modes of action. Field studies were conducted in 2015 and 2016 at Concord, NE and in 2017 at Concord and Clay Center, NE to evaluate the effects of PRE herbicides on critical time for weed removal (CTWR) in soybean. The studies were laid out in a split-plot arrangement of 14 treatments (2 herbicide regimes and 7 weed removal times), with eight (2015), and four replicates (2016 and 2017). The 2 herbicide regimes were: No PRE and PRE application of sulfentrazone plus imazethapyr at across all years. The seven weed removal times across all years and locations were: V1, V3, V6, R2 and R5 soybean growth stage, as well as weed free and weedy season long. There were statistical differences between the three years; therefore, data was presented by year. In 2015, CTWR (based on 5% acceptable yield loss) started at V1 soybean stage without PRE herbicide, while the PRE application of herbicide delayed CTWR to V5 soybean stage. The CTWR in 2016 started at V3 soybean stage without PRE herbicide, while the PRE herbicide delayed the CTWR to R1 soybean stage. In 2017, CTWR started at V1 soybean stage without PRE herbicide, while the application of PRE herbicide delayed the CTWR to V7 soybean stage. These results clearly showed the benefit of using PRE herbicides to reduce the need for multiple applications of glyphosate, and provide additional mode of action for combating glyphosate resistant weeds.

**Efficacy of 2,4-D, dicamba, glyphosate and their mixtures on hedge bindweed control**Aleš Kolmanič<sup>1</sup>, Robert Leskovšek<sup>1</sup>, Mario Lešnik<sup>2</sup><sup>1</sup>Agricultural Institute of Slovenia, LJUBLJANA, Slovenia<sup>2</sup>Faculty of Agriculture and Life Sciences, MARIBOR, Slovenia

Hedge bindweed (*Calystegia sepium* L.) is a wide spread perennial weed species with extensive rhizome development and vigorous regeneration capacity from the root system. The aim of this study was to evaluate hedge bindweed long term control (damage to root system) with various herbicide tank mixtures and application timing in comparison to the application of single active ingredient. A pot experiment with randomized complete block design was conducted in 2015, where the efficacy of glyphosate 2,4-D and dicamba, their tank mixtures and delayed split applications for hedge bindweed control was determined. Two different glyphosate (isopropylamine and ammonium salt) forms were applied at the rate of 2000 g a.i./ha, 2,4-D DMA (dimethylamine salt) was applied at 500 g a.i./ha and dicamba at the rate of 500 g a.i./ha with a spray volume of 250 l/ha. Sequential application was performed 12 or 24 hours after the first application. Efficacy was determined eight months after treatment with visual rating and by weighing of shoot and rhizome biomass of treated and non-treated plants. 2,4-D showed the highest overall efficacy when applied alone, even when relatively low dose was used (91–95% and 75–82% for shoot biomass and rhizomes, respectively). Dicamba reduced hedge bindweed shoot and rhizome biomass by 66–87% and 67%, respectively. Glyphosate control did not differ between two formulations and ranged from 82–85% and 39–47% for shoot and rhizome biomass. A possible moderate antagonisms between the glyphosate and 2,4-D, and between the glyphosate and dicamba were observed when applied as tank mixtures. Efficacy of tank mixtures was generally lower compared to 2,4-D or dicamba applied alone. The highest efficacy on rhizome biomass reduction with sequential applications were observed when glyphosate was applied first at 2000 g/ha, followed by 500 g/ha dicamba (93% efficacy) or 2,4-D (84–94% efficacy) 12 hour later.

### **IWMPRAISE - an EU HORIZON 2020 project on Integrated Weed Management**

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IWMPRAISE is a new five-year EU Horizon 2020 project on integrated weed management (IWM) with 37 partners in eight European countries. The main objective of IWMPRAISE is to demonstrate that adoption of IWM will support cropping systems that are agronomically and environmentally more sustainable and more resilient without jeopardizing profitability or the steady supply of food, feed and biomaterials. IWMPRAISE will develop, test and assess management strategies delivered across cropping systems for four contrasting crop groups: narrow-row annual crops, wide row annual crops, perennial herbaceous crops and perennial woody crops. The specific objectives are to i) quantify and address current socio-economic and agronomic barriers to the uptake of IWM ii) design, evaluate and optimize novel alternative weed control methods and create a 'tool box' of validated IWM methods iii) assess the short- and long-term agronomic performance and environmental and economic sustainability of IWM strategies and iv) make results available to end users. One WP is devoted to understanding end users' perception of IWM and barriers to uptake of new knowledge while another WP will focus on the interface between weed management and tillage. Novel IWM strategies will be developed within national clusters where all stakeholders are represented. The development of IWM strategies are supported by research activities delivering practical knowledge and novel tools for weed control as well as tools for assessing and disseminating the novel strategies. IWMPRAISE will provide advances beyond the state-of-the-art within several research areas ensuring that the overall goal of the project, to provide practical solutions to the end users, will be fulfilled. IWMPRAISE combines R & D activities, providing the tools for developing IWM strategies, with activities that adopt the »interaction innovation model« involving end-users and other stakeholders in a partnership with public research institutes and private SMEs.

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**Exchange rate between herbicide dose and crop competition for control of ambrosia**

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Weeds surviving an herbicide treatment in a competitive crop will undergo stress due to both crop competition, and the herbicide efficacy. The impact of each of these factors was studied using the target neighbourhood design with spring barley as the crop and *Ambrosia artemisiifolia* as the weed. Different scenarios for ambrosia-crop competition were established by transplanting one ambrosia plant (growth stage BBCH 12) into the center of each pot (6.5 L) surrounded by five different densities of spring barley (0 to 600 plants/m<sup>2</sup>) at two different growth stages (BBCH 12 and 21). Six different doses of mechlorprop-P (MCP) (from 0 to 1200 g/ha) were applied one week after transplanting. The ambrosia plants were harvested at crop maturity. A non-linear regression model was fitted to the fresh weight data, and the ED50 and ED90 values were estimated for each crop density. In addition, the crop densities providing 50 and 90% reduction of ambrosia biomass were estimated for each herbicide dose. In the scenarios of crop and weed emerging at the same time, the competitive ability of 340 barley plants per m<sup>2</sup> in untreated was equivalent to the efficacy of 225 g/ha MCP on ambrosia growing with no crop competition. However, if barley emerged 10 days before ambrosia only 50 barley plants were needed to obtain the same reduction in biomass. These results illustrate that ambrosia is highly affected by crop competition. Establishment of a dense crop cover is a good first step when using IPM for ambrosia control.

### **What is the best alternative to herbicides? A case study from a Swiss vineyard**

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The main challenge to viticulture without herbicides is the management of vine rows. This study aims to compare the most common row management strategies, taking into account agronomic and environmental factors.

The trial occurred in a Swiss vineyard previously weeded with herbicides. The experimental design was a randomized block with five treatments and four replications per treatment. The five row (inter-vine) management techniques were: i) herbicides as a control (alternating between glyphosate and glufosinate); ii) mowing of vegetation; three types of mechanical weeding: iii) rotary brushes, iv) blade and v) disc harrows alternated with a finger weeder. Vegetation, in conjunction with soil properties and vine and must qualities, was assessed over the 2017 season.

The herbicide, blade and disc harrows/finger weeder techniques were the most effective since they maintained the lowest mean soil plant cover in summer (18%, 23%, and 24% respectively), followed by the rotary brushes (47%) and mowing treatment (66%). In terms of biodiversity, the herbicide treatment had a species richness of six weed species compared to nine for mechanical weeding techniques and eleven for mowing. Vines treated with all techniques except herbicides had similar nutritional values which were lower than for the herbicidal treatment (yield, nitrogen nutrition, leaf water potential, net photosynthesis and stomatal conductance). This is likely due to a high competition for resources where rotary brushes were used and in the mowing plots. Moreover blade and disc harrows/finger weeder might destroy shallow vine roots.

Altogether, our results show that during this first year of reconversion, the four row management strategies without herbicides had a similar impact on vine, but mowing provided additional ecosystem services (e.g. soil protection and soil biodiversity).



## **Mental modelling of European farmers' decision making processes regarding Integrated Weed Management**

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Integrated Weed Management (IWM) typically involves complex risk management decisions. It comprises preventive and control measures that require decisions on crop choice and sequence, cover cropping, fertilisation, cultivation type and frequency. IWM can therefore not be considered a set of weed control tactics alone, it is a complex system approach in which many different risks and benefits need to be considered. Many farmers have not embraced IWM practices despite proven to mitigate weed problems and increasing the sustainability of weed management.

Within the project IWM PRAISE, we defined a framework for IWM applicable in several cropping systems. Five different classes or pillars are distinguished for integrated weed management, which are important to make an informed decision on what tactics to combine into a weed management strategy that manages weed populations at a time scale covering the current growth season.

Weed management experts from the Netherlands, Denmark, UK, France, Slovenia, Italy and Spain were interviewed to add expert based knowledge to the IWM framework. Furthermore, farmers from all above countries were interviewed to identify the farmer's knowledge, thinking and decision process regarding IWM strategies and tactics covering different cropping systems. Analyses of the differences between expert's and farmer's thinking about IWM and the differences in knowledge and use of IWM strategies and tactics between the different cropping systems will be presented, together with the IWM framework. Knowledge on farmers' thinking and decision-making processes can be used to guide research activities tailored to the farmers' needs. The research is carried out in the IWM PRAISE EU H2020 project.

## Crop rotation and fertilization effects on weeds in rice based cropping systems in Madagascar

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In the Mid-West region of Madagascar, yields in upland rice remain low due to both low soil fertility and high weed pressure in fields. Increasing managed biodiversity inside crop rotation may be an option to reduce weed pressure in cropping systems.

The effect of managed biodiversity was studied in a field experiment carried out in Ivory (19°33'18.90«S, 46°24'53.08«E), with a randomized block design with four replications during two years. Three rainfed rice based rotations (Rice // Groundnut = RA, Rice // Sorghum + *Vigna unguiculata* = RSV, Rice//*Mucuna cochinchinensis* + *Crotalaria spectabilis* = RMC) combined with two levels of fertilization (Low Fertilization = manure vs. High Fertilization = manure + fertilizer) were compared to a rainfed rice monoculture. Each crop or crop mixture in the rotation was grown every year on plots measuring 45.9 m<sup>2</sup>. Each year, weed and rice biomass were measured at each weeding date. Rice biomass was also measured at flowering and harvest. Rice yield and its components were measured at harvest. Weed flora was observed the second year to analyse potential change in weed communities according to fertilization and/or crop rotation.

The first year, weed biomass and rice yield were higher in highly fertilized treatments than in lowly ones. In highly fertilized treatments, weeds did not affect yield and weed biomass was positively correlated to rice yield. On contrary, fertilization had no effect on weed biomass the second year. The highest and lowest weed biomass were observed on RA and RMC treatments respectively. Lowest rice yields were observed in RSV and RA treatments, in highly and lowly fertilized treatments respectively. In lowly fertilized treatments, weed biomass reduces significantly rice yield. Finally, rotation had more impact than fertilization on weed flora.

Biodiversity may have positive effect on cropping systems performances but it depends largely on the introduced species.

## **Tillage and crop rotation effect on weed control in the stand of winter wheat**

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Winter wheat is the most important grain crop in Lithuania. Over the period 2014-2015 a series of field experiments were conducted in Dotnuva (Lithuania) on *Endocalcari-Endohypogleyic Cambisol* with the aim to reveal the suitable preceding crops of short crop rotations by applying contrasting tillage systems on weed community composition in the stand of winter wheat. The experiment consisted of two tillage treatments: mouldboard ploughing (CT) and stubble cultivation (RT), and two short crop rotations: 1) spring oilseed rape - spring barley - winter wheat, 2) winter wheat - winter oilseed rape and 3) winter wheat grown as monoculture. It was revealed that weed densities varied between rotations. The number and dry weight of weeds was recorded at the winter wheat BBCH 83. The assessments were made using a 0.25 m<sup>2</sup> frame by collecting weeds in four places for each replication. In winter wheat stand in all crop rotations the density of *Chenopodium album* L. and *Sinapis arvensis* L. was high, but *Fallopia convolvulus* (L.) Love, *Taraxacum officinale* F. H. Wigg., *Tripleurospermum perforatum* (Merat.) M. Lainz., *Capsella bursa-pastoris* (L.) Medik. and *Stellaria media* (L.) Vill. was less numerous. CT decreased the number of weeds on average by 94 % and dry mass of weeds by 57 % in all crop rotations as compared to RT. The number of weeds under CT depended on the interval of winter wheat returning to the same field. After two or after one year duration, the weed incidence consistently declined - 73 and 95 weeds m<sup>-2</sup>, respectively. Dry mass of weed under RT was the highest (485 g m<sup>-2</sup>) in the two-course crop rotation, while in the monoculture of winter wheat it reduced to 386 g m<sup>-2</sup>. In the three-course crop rotation the mass of weeds was the least and amounted to 280 g m<sup>-2</sup>.

## **Efficacy and Economic of Weed Control in Soybean Production**

Edita Stefanic, Darko Dimic, Ivan Stefanic  
Faculty of Agriculture, OSIJEK, Croatia

Row spacing and length of weed interference are variables that can have a significant impact on net returns of soybean producers. Row spacings affect the time of canopy closure, and therefore influence both the crop and weeds. Field studies were conducted in 2014, 2015 and 2016 at Vukovar (lat 45.345°N, long 19.001°E), North-East Croatia to determine the effect and economics of weed control in narrow (25 cm) and wide (50 and 70 cm) row spacings on soybean growth and yield. Experiments were established in a factorial arrangement of treatments in a split-plot design. Soybean row spacing was the main plot and six weed control options as subplots with four replication of each treatment. A naturally occurring population of mixed weed species was utilized to obtain appropriate duration of weedy periods.

A Monte-Carlo simulation model has been constructed to investigate the probabilities of profit from controlling weeds at different row spacing and management treatment. The economic analysis was based on variable cost of production.

Common lambsquarters (*Chenopodium album* L.) and johnsongrass (*Sorghum halepense* L.) were the major weed species present each year and comprised of more than 75% of total weed biomass at the late season harvest in each of study years. Soybean yield loss varied across years, therefore data were presented separately for each year of the experiment.

A pre-em herbicide treatment followed by mechanical cultivation in 70 cm row spacing provided excellent weed control in all investigated years and had the highest gross margin.

This study confirmed that soybean row spacing significantly influenced crop-weed interference, had different gross margin and therefore required different weed management programs.

## How to manage increasing problems with *Echinochloa crus-galli* in northern Europe

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*Echinochloa crus-galli* has expanded in recent years from along the coastal areas of the Oslofjord to beyond 60 °N in Norway. Well-adapted biotypes competes well in spring sown crops. Yield losses in less competitive vegetable crops are much higher than in cereals due to infestations. *E. crus-galli* is difficult to control even with effective herbicides at hand. The main objective of the new project ECRUSLI is to find effective direct and preventive control measures against *E. crus-galli* in Norwegian cereals, potatoes and vegetables. Here we present the first results from the cereal trials (randomized complete block design with 3-5 replicates). We investigated herbicides and application techniques at different times in spring cereals in three trials. Propoxycarbazone-sodium (early) and pinoxaden (late with or without crop tilter) proved to give good control. In two of these field trials on untreated plots and in one pot trial with various populations, *E. crus-galli* was grown with or without cereals. The results showed that the total emergence of *E. crus-galli*, aboveground biomass and number of produced seeds were reduced on average by 25, 70 and 95%, respectively, with a crop present compared to crop-free conditions. The time of emergence was not influenced by crop. In an experiment in spring oats we investigated the biological control rate of *E. crus-galli* seeds. Seed cards, without or inside cages excluding larger predators like rodents and birds, were exposed to naturally occurring seed predators at four periods. Seed removal rate was higher from cards without (30%) than inside (5%) cages. For the former, the highest rates, about 50%, occurred before harvest. Results indicate that predators larger than insects are important in biological control of *E. crus-galli*. Acknowledgements: Funded by Norwegian Research Funding for Agriculture and Food Industry (The Research Council of Norway project no. 267700) and project partners.

### **Weed control in the production of black locust seedlings**

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The presence of weeds in forest nurseries is the main limiting factor of successful production of planting materials. Application of herbicides reduces weed spreading, especially in the initial phases of forest seedling development when the unfavourable impact of weeds on seedlings is the highest. Mechanical weed control is often inefficient and expensive because it has to be repeated several times during the growing season. Such measures are increasingly being replaced by more efficient measures of weed control. During the 2016-2017 experiments were established in order to investigate the possibility of weed control by herbicides in production of black locust seedlings. Experiments were conducted in the Centre for Nursery Production of Forest Seedlings – Brcko, Bosnia and Herzegovina. The investigated post-emergence herbicides imazethapyr (Pivot), imazamox (Pulsar 40) and cycloxydim (Focus ultra). Experiments were established using randomized block design in three replicates. The efficiency and phytotoxicity of herbicides was evaluated in 2, 4, 6 and 8 weeks after treatment. The results showed that used herbicides were effective in the control of weeds. With the use herbicide imazethapyr achieved very good efficacy in control broadleaf weeds. (82.0% - 96.0%). The lower efficacy was achieved by applying the herbicide imazamox (79.00% - 89.5%). Herbicide cycloxydim was very effective in control of grass weeds (95.0% - 99.0%). Herbicides imazethapyr and cycloxydim did not have phytotoxic effect on seedlings black locust but herbicide imazamox caused phytotoxic effect.

## Implementation of control strategies against *Cyperus esculentus* in practice

Judith Wirth, [Christian Bohren](#)  
Agroscope, NYON, Switzerland

Yellow nutsedge (*Cyperus esculentus* L.) is a problematic weed in Switzerland mainly in farming regions where vegetable and field crops are grown next to each other, implicating land exchange between vegetable and crop farmers. Control strategies include preventive measures for stopping the unintentional spread of tubers as well as approaches adapted to the infestation level in each field.

In 2016 a Swiss national four year project was started by several partners from practice and research in order to develop a national control strategy for yellow nutsedge. It includes two sub-projects: a) field trials in infested regions throughout the country in order to evaluate control strategies and b) workshops with farmers, contractors, marketing organizations and cantonal plant protection services on various topics such as dispersal and unintentional spread of tubers.

Farmers provided a total of 21 »pilot-fields« for yellow nutsedge control on farm. In close collaboration with cantonal advisors most of these farmers adapted their cultivation methods with regard to better control efficiency. The strategy »late maize drill« was applied in most cases: in early spring a stale seedbed is prepared, followed by an additional soil cultivation to destroy yellow nutsedge at the 2-5 leaf stage. Immediately before late sowing (end of May) 2 l/ha Dual Gold® (960 g/l s-metolachlor) is incorporated. The fields are accompanied during the four year project period and the control effect is assessed by sampling and counting the number of yellow nutsedge tubers in the soil each autumn from 2016 to 2019. After the first year of control measures a significant decrease in tuber numbers could only be observed in two fields. Tuber sampling over the next few years will allow to evaluate whether yellow nutsedge can be effectively controlled with the proposed control strategies.

### **Co-design of a Decision Support System for integrated weed management**

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Each cropping practice affects weed dynamics depending on pedoclimate or time-step. Weed management must be reasoned at the cropping-system scale and multiperformant cropping-system design is difficult. Decision Support Systems (DSS) for farmers and crop advisors are essential, and to make these tools operational and utilized in practice, end users must be involved when designing the tools. Our aim was to test a DSS prototype with end users to identify its strengths and weaknesses. The prototype consisted of (1) a »design guide«, i.e. a decision tree visually linking combinations of cropping practices to performances in terms of weed harmfulness for crop production and weed-borne biodiversity, (2) a »fast prediction tool«, i.e. a random forest predicting these performances from cropping-practice combinations proposed by users. Both were built from a cropping-system database simulated with the mechanistic weed dynamics model FLORSYS.

Workshops were conducted in 2017 with five advisors from Champagne-Ardenne (France). The first workshop aimed to design innovative cropping systems adapted to different regional production situations and to choose weed-impact indicators to evaluate the systems. In-between workshops, the systems were simulated with FLORSYS to estimate indicators. In the second workshop, the simulation results and the design guide were presented, testing different output formats. After the workshops, the prediction tool was tested by the advisors via an online R-shiny application. Feedbacks on the application's use were collected via an online questionnaire.

Several improvements were suggested, especially for synthetically describing a cropping system. The DSS promoted discussions among advisors, especially when confronted with surprising results (e.g., alfalfa did not increase field infestation). The workshop format was adapted to improve the DSS and to demonstrate its usefulness.

Funding: INRA (EA and MIA divisions), the French project CoSAC (ANR-14-CE18-0007) and the Burgundy Region. Thanks to crop advisors of the Chambers of Agriculture of Haute-Marne and Aube (France)



**S12-O Weed Ecology  
ORAL PRESENTATIONS**

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### **What is the contribution of the residual weed floras to reduce nitrate leaching?**

Delphine Moreau, Olivia Pointurier, Bernard Nicolardot, Nathalie Colbach  
INRA, DIJON, France

Integrated crop protection tolerates residual weed floras if they are not harmful for crop production. Weeds are often solely considered with a negative viewpoint, but they may also provide beneficial services for agroecosystems, such as plant biodiversity promotion and trophic resources for other organisms such as pollinators. The role of residual weed flora as nitrate catchers, during the summer fallow, has seldomly been assessed, in spite of the high potential of some weed species to take up nitrogen. The present study aimed to (1) develop an indicator to account for the potential role of weed floras as nitrate catchers at the field scale, and (2) calculate this indicator from the outputs of a weed dynamics model (FlorSys) in order to analyse, with simulations, whether weed floras may, in some situations (to be identified), significantly contribute to reduce potential nitrate leaching, while limiting negative impacts on crop production. When developing the indicator, we considered that the potential of weed floras as nitrate catchers depended on both the 'nitrophilic' level and the plant leaf area of the species present in the weed floras, during the period running from the harvest of the preceding crop to the beginning of water drainage. This indicator was used to predict the potential of nitrate catching by weed floras in cropping systems from six regions from France and one from Spain which were simulated with the FlorSys model. Data analysis (under progress) will determine trade-offs between this indicator and other indicators of weed harmfulness for crop production and weed contribution to plant and functional biodiversity. It will help to identify in which production situations residual weed floras can play a significant role in preventing nitrate leaching, while minimising negative weed impacts, such as crop yield loss and harvest pollution by weed seeds. Funding: INRA, CoSAC project (ANR-15-CE18-0007).

## **Improving the ecological impact of maize cropping through sown wildflowers using strip-tillage**

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The enlargement of maize cropping within, among others within energy cropping systems, is widely discussed as major threat to weed and wildlife biodiversity in Germany. The two main reasons for the negative impact on various biodiversity compartments are the late soil tillage date and the lack of weeds, flowers or vegetation structure at the early growing stages of maize.

We developed a new cropping system for maize based on strip-till and band spraying technologies. Wildflowers were sown between the future maize rows after harvest of the last main crop in the year before. With these wildflower strips we wanted to avoid late tillage and provide flowers and vegetation structure on maize fields. We have conducted field trials at two sites in Germany, where the agricultural feasibility, ecological and yield impacts have been analysed. Within these trials the following five factors have been tested: i.) two wildflower mixtures, ii.) three cover crops for establishing the wild flowers, iii) two widths for the wildflower cover crops, iv) two widths for the wildflower strips, and v.) two widths for chemical plant protection treatments.

We present the results of these field trials with special regard on the agricultural feasibility and the ecological effects of the new cropping systems using conventional maize cropping as reference. The result show the successful establishment of the wildflowers strips between the maize rows. Flowering diversity and duration was ten times higher than in conventional maize crop stands and lasted the whole cropping season. Weed species diversity was elevated 3-4times in the new cropping systems. Positive implications on pollinators could be proved. A yield reduction of 30% was observed, but it could not be related to the wildflower competition alone. The management of spontaneous weeds needs some further improvement.

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**The response of weed and crop species to shading: measurement and prediction from traits**

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Crops often compete with weeds for light, and choosing competitive crop species is a major lever for managing weeds. The present study aimed to (1) measure the range of species parameters that drive light competition in contrasting crop and weed species of temperate European arable crops, (2) relate the parameter values which are difficult to measure to species traits that are easier to access, by establishing trait-parameter relationships, (3) integrate the measured parameter values into FlorSys which simulates weed dynamics and crop canopy growth in virtual fields over the years with a daily time step, and (4) run simulations to investigate which crop and weed parameters are linked to weed harmfulness for crop production. 33 weed species and 25 crop species were investigated. Parameters driving initial growth were measured in optimal light and nutrient conditions in a greenhouse with automatic non-destructive measurements. Parameters describing potential morphology in unshaded conditions were measured on plants grown in optimal light and nutrient conditions in garden plots and harvested at 4-5 stages during plant cycle. Shading response was measured by comparing potential morphology to that of plants grown under shading nets in these same gardens. All parameters could be predicted from seed (weight, lipid content...), plant (epigeal vs hypogeal growth, form...) and general traits (clade, base temperature, plant lifespan, legume vs non-legume...). Crop species that decreased weed impact the most grew fast after emergence (high relative growth rate RGR), had thinner larger leaves (high specific leaf area SLA), were wide rather than tall, and allocated biomass preferentially to stems vs leaves. Harmful weed species presented a large leaf area at emergence and strongly responded to shade, by increasing their height, leaf biomass and area per plant biomass unit.

Funding: INRA, CoSAC project (ANR-15-CE18-0007), ReMIX (EU-H2020-727217)

**Defining the habitat niche of *Alopecurus myosuroides* (black-grass) at the field scale.**

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*Alopecurus myosuroides* Huds. (black-grass) is one of the most common grass weeds of winter cereals in north-west Europe (Holm et al., 1997) and is particularly problematic in the UK. *Alopecurus myosuroides*, like many weed species, grows in patches within fields. The locations of these patches can be influenced by the environment. This presents an opportunity for precision management through patch spraying.

We surveyed five fields in commercial winter wheat production with a range of soil types using a nested sampling design. This type of design allowed us to study scale dependent relationships between *A. myosuroides* densities and soil properties. We recorded both *A. myosuroides* seedlings in autumn and heads in summer. We also measured soil properties at those sampling locations.

We found that the patches of heads within a field were smaller than the seedling patches, suggesting that techniques for patch spraying based on maps of heads in the previous season could be inherently risky. We also found that the location of *A. myosuroides* patches within fields can be predicted through their relation with environmental properties, particularly soil moisture, soil pH, and topography. These relations are consistent across fields on different soil types.

This improved understanding of the relations between soil properties and *A. myosuroides* seedlings could allow farmers to use pre-existing or suitably supplemented soil maps already in use for the precision application of fertilizers as a starting point in the creation of herbicide application maps.

The scale-dependent correlations that provide the strongest links between *A. myosuroides* counts and soil properties are most often found at coarse scales corresponding to the scale of operation of most farm machinery and as such decisions could be made at a relevant scale for management if patch spraying were to be implemented based on soil maps.

## **Weed communities show differential response to management intensity and landscape diversity in European vineyards**

Silvia Winter<sup>1</sup>, Nicole Penke<sup>1</sup>, Monika Kriechbaum<sup>1</sup>, Daniela Popescu<sup>2</sup>, Ángel Lora<sup>3</sup>, Rafael Sánchez de la Cuesta<sup>3</sup>, Gema Guzmán<sup>4</sup>, José Manuel Cabezas<sup>4</sup>, Jose Gomez<sup>4</sup>, Vincent Jung<sup>5</sup>, Albin Fertil<sup>5</sup>, A. Schneider<sup>5</sup>, Simon Chollet<sup>5</sup>, Maria Comsa<sup>2</sup>, Sophie Kratschmer<sup>1</sup>, Muriel Guernion<sup>5</sup>, Adela Hoble<sup>6</sup>, Annegret Nicolai<sup>5</sup>, Johann G. Zaller<sup>1</sup>

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Weed communities in vineyards are affected by a range of different management measures which either try to maintain vegetation cover in the inter-rows or eradicate any competition between vines and other plants. Vegetation within vineyards provides important ecosystem services, such as soil erosion mitigation, carbon sequestration and the provision of habitat and food resources for natural enemies of pests. However, dense vegetation cover and the presence of competitive weed species can also lead to increased water (and nutrient) competition between spontaneous vegetation and vines during dry periods. In the BiodivERsA project VineDivers »Biodiversity-based ecosystem services in vineyards: analysing interlinkages between plants, pollinators, soil biota and soil erosion across Europe«, we analysed the effects of three different management intensities (bare soil, permanent or temporary vegetation cover) on above- and below-ground biodiversity and the associated ecosystem services across Europe. In this paper, we present the results of the plant diversity survey from 81 Austrian, French, Romanian and Spanish vineyards. We recorded plant diversity, biomass and species composition in four plots within each vineyard. In addition, we calculated Shannon landscape diversity index (SHDI) and further landscape parameters within a landscape circle of 1.5 km diameter around the vineyards. Species diversity of the most intensive bare soil management system was lower than in vineyards with permanent vegetation cover; however, this difference was only significant in France. The differences of species diversity between temporary and permanent vegetation cover were slighter than expected. Different management systems favoured specific functional traits and life strategies, e.g. a reduced leaf area and higher coverage of annual and ruderal species in bare soil vineyards. These traits influence biomass production and consequently various ecosystem services like soil erosion mitigation or carbon sequestration. Landscape parameters like SHDI and percentage of vineyards influenced plant diversity only in some countries.

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## **EU Herbicide Regulation: Protection goals for non-target plants and in-crop protection of beneficial weeds**

Joanna Davies, Leslie May, Adam Russell, Antony Seville, David Stock  
Syngenta, BRACKNELL, United Kingdom

EFSA's Scientific Opinion addressing the state of the science on the risk assessment of plant protection products for non-target terrestrial plants (NTTPs) was published in 2014. The Opinion defines non-target plants as all plants growing outside fields and those growing within fields that are not the target. It also states that non-crop plants growing in in-field areas provide ecosystem services including food web support, aesthetic value, genetic resources and endangered species, which require protection from the adverse effects of plant protection products. As such, The Opinion advocates the protection of plant species growing in-field that under current agricultural practice would be considered target weeds growing in the crop. Since 2014, options for protecting ecosystem services provided by non-crop plants growing in-field, have been discussed in various stakeholder workshops and publications. Proposed options include (a) compensation mechanisms whereby designated areas are set aside specifically for NTTPs, and (b) beneficial weed protection, which involves the control of those weed species that compromise crop yield while not affecting other species that are considered to have ecosystem service value. To inform this debate, the feasibility of beneficial weed protection will be considered from three perspectives: (a) issues relating to the definition of beneficial weed species (b) potential agronomic consequences of protecting beneficial weeds and (c) challenges facing the discovery and registration of selective herbicides. The issues outlined in this presentation will illustrate that the feasibility of protecting weeds in-crop requires investigation of complex species definitions and agronomic consequences while the discovery and availability of new herbicides with the required selectivity is considered unlikely, particularly under the current regulatory framework in the EU.

## **Do weed species tend to occur more often where neighbors have similar trait values?**

Eva Hernández Plaza<sup>1</sup>, Valle Egea Cabrero<sup>1</sup>, Jose Luis González Andujar<sup>1</sup>, Fernando Bastida<sup>2</sup>

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Management practices and other environmental conditions may represent important filtering processes and constrains on the assembly of weed communities. This could be in accordance with evidences showing weed community weighted mean trait values (CWMs) varying along environmental gradients. If this is the case, CWMs may represent the optimal weed strategies for the conditions of a certain field. Following this, weed species whose trait values are similar to the local (community) values will tend to have a higher fitness in a specific field. To test this hypothesis, we sampled weed species abundance in ten 1 m

<sup>2</sup> plots across 23 winter cereal fields in Andalusia (Spain) in 2017. Fields were either conventionally or organically managed, representing a gradient of contrasting management intensity in terms of fertilization and herbicide use. We obtained data from bibliography on the value of four traits for each of the 146 species recorded: maximum height, leaf dry matter content, seed mass and month of flowering onset. For each trait and species (found in at least 10% of the sampled fields), we calculated the absolute difference between the species trait value and the CWM value for each plot ( $|\Delta\text{CWM}|$ ). An important consideration is that in each case, the focal species was excluded when computing the CWM. The number of plots per field in which a species was recorded (OCC) was used as a proxy for its fitness. We related OCC and  $|\Delta\text{CWM}|$  values for each species and trait by linear regressions. A negative slope is interpreted as the species supporting our hypothesis. Finally, we used a randomization procedure to determine whether the number of species with significant negative slopes is greater than expected by chance. We show whether this differ among the traits analyzed.



**S12-P Weed Ecology  
POSTER PRESENTATIONS**

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## **Are tillage system and annual weather determining the functional structure of weed communities?**

María Remedios Alarcón Villora  
IMIDRA, ALCALÁ DE HENARES, Spain

Tillage is one of the main practices for weed management and its effects on weed biodiversity have been studied for decades. However, a better insight on the processes determining shifts in weed communities would be gained from a trait based approach. Here we ask whether the type of tillage system and the variability in climatic conditions may explain differences in the functional structure of a weed community. To do that we used data on weed abundance from an experiment in which three types of non-inversion tillage systems were compared: no-tillage, subsoil tillage and minimum tillage. To evaluate the effect of inter-annual climatic variability weeds were recorded throughout nine years.

We calculated the community weighted mean (CWM) and the mean pairwise distance (MPD) of six response traits (maximum height, SLA, seed weight, growth habit, dispersal mode and seed longevity index). We also computed a multi trait MPD index as a proxy of the community global functional diversity. Species traits were measured in a near fallow area. We considered three climatic variables (precipitation, average temperature and the number of frost days) to characterize climate annual conditions.

Tillage system affected the multitrait and the growth habit MPD and the dispersal mode CWM. Precipitation was associated with SLA, seed weight, longevity index and growth habit MPD, and to seed weight and dispersal mode CWM. Temperature was related to height and seed weight MPD, and to seed weight and dispersal mode CWM. Finally, the main effects of frost were related to dispersal mode CWM.

Annual climatic conditions are the main factors determining weed communities from a functional point of view, with tillage system playing a minor role. This could be related with the long history of tillage practices on these communities and with the great climatic variability typical of Mediterranean regions.

### **Evolution of weeds in maize and winter cereals in Transylvania**

Ileana Bogdan, Teodor Rusu, Adrian Ioan Pop, Paula Ioana Moraru, Stefania Claudia Gâdea  
University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, CLUJ-NAPOCA, Romania

Inventory of crop weeds provides farmers with valuable information on species distribution and abundance, which are absolutely necessary both for the success of IWM strategies on farms and for the discovery of invasive species in their area or missing species.

The study presents the comparative results of 3 series of weed mapping from winter cereals and corn crops in the central area of Transylvania. These crops predominate in the area with a major influence on the cultural hygiene of farms.

The purpose of this research was to determine the evolution of the floral weed spectrum from the predominant crops in the center of Transylvania over a period of 30 years. The causes that have contributed to the weedy characteristics over the years have been established too. Three different weed mapping periods were used at the same locations. The first set of data was extracted from the national weed mapping databases from arable lands in Romania, made in 1986 at national level. The second set of data was obtained by successive weed inventories during the period 1998-2001 carried out within a research project conducted by the Romanian Academy of Agricultural and Forestry Sciences. The third set of data was obtained by successive weed inventories in 2014-2016 through own research projects.

Numerical Quadrat Square method were used for weed sampling, in 26 localities in Cluj County. 46 stations were delineated, covering 11 types of soils and 152 plots for samplings.

The data analysis demonstrates a decrease in the total number of weed species present in both groups of crops, as well as significant variations in floral spectrum. There have been remarkable species whose evolution is spectacular, while other species with high frequency in 1986 are currently very poorly represented.

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## **Merging European arable weed survey datasets for comprehensive analyses. Case study: Species Distribution modelling**

Jana Bürger

University Rostock, ROSTOCK, Germany

During previous EWRS meetings, a recurrent proposition was made to the participants to combine the various weed survey datasets previously collected by weed scientists all over Europe. A joint dataset could facilitate analyses on weed communities and the underlying factors or patterns on a larger spatial scale than so far.

Despite interest to contribute to such a project, many reserachers have called for case studies to prove the benefits of the effort to collect and join data bases. Here we present such a case study.

We build Species Distribution Models (SDM) for ten important European weed species, based on climate and vegetation data from three different sources. This kind of model can help for example to estimate future changes in the distribution of a species following climate change, or more specifically to assess the probability of establishment of emerging weeds in various crops and regions.

SDM are often built with easily available data, like the large collections generated by vegetation scientists. Regarding agronomic questions, the results may not be very meaningful, because the data contain species occurrences from various sorts of ecosystems. In contrast, weed survey datasets usually contain information on the agronomy of the fields.

Data sources for the case study are:

- 1) GBIF (general repository, vegetation data from all type of habitats),
- 2) GBIF as in 1), but only from regions of Europe where the species was evaluated an important weed in certain crops by experts,
- 3) joint weed surveys (possibly separated for different crops or sowing periods according to agronomic data).

The quality of the models is evaluated against a test data set. We hypothesise that model quality in respect to the occurrence of a species in a certain crop will increase with the increased accuracy of the used vegetation data in respect to the agronomic context.

**When it comes to tillage, timing matters and drives weed communities**

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Tillage is a foundational management practice in many cropping systems to manage weed seedbanks and reduce reliance on herbicides for weed management. Tillage alters established weed communities but can also stimulate weed seed germination and emergence. Hence, viewed within a community assembly framework tillage is a strong assembly filter that can either constrain or advance the membership of species within the subsequent weed community. At four locations in the Northeastern United States, twelve treatments of a single primary tillage (10-cm depth) event at 2-week intervals over the course of the growing season were compared using a randomized complete block design with four replications. The emergent weed community in each treatment replicate was quantified 6 weeks after the tillage operation. We identified three main periods of tillage timing that resulted in similar communities. Across all sites, total weed density tended to be greatest and weed evenness tended to be lowest when soils were tilled early in the growing season. The timing of tillage explained approximately 50% of the weed community variability. Weather conditions, mainly growing degree days, but also precipitation occurring before tillage, were important factors and could improve our ability to predict the impact of tillage timing on weed community assemblages. No single trait or combination of traits were consistently associated with species-by-tillage time groupings across locations; however, within each location several traits were associated with particular groups of species, including seed length, seed weight, cotyledon type, life span, ploidy level and photosynthetic pathway.

## **Analysis of sampling errors for small-scale sampling of weed populations**

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An aggregated weed distribution could allow for significant herbicide savings if the site-specific weed management (SSWM) will be adopted in the practice. This, however, will require reliable and detailed mapping of weed populations. A quantification of sampling precision is needed for small-scale sampling strategies, which will be applicable in SSWM. In this work, an effect of sample size on sampling error was studied in two sub-field areas infested with *Galium aparine*. The mapping was carried out during March or April just before postemergent herbicide treatment. Plots of 54 x 48 m and 60 x 48 m were marked and position of all *G. aparine* individuals was recorded using RTK (Real Time Kinematics) navigation system. Mapping cells of 3 x 3 m size were arranged over experimental plots and various sampling intensities of *G. aparine* populations were then simulated in these cells using increasing numbers of sampling quadrats of 0.25 x 0.25 m size. Sampling intensities ranged between 0.06 % and 100% of the total area. The Mean Absolute Percentage Error (MAPE) was calculated and plotted against individual sampling intensities. This regression was fitted with non-linear model in R. Both experimental plots showed relatively low mean density of *G. aparine* (2.98 and 1.01 plants m<sup>-2</sup>) and spatial distributions of plants were patchy in both cases. MAPE values decreased inversely to the sampling intensity. Based on fitted model, it was calculated, that maintaining the MAPE below 30 % would require sampling of 30.2 % of total area for lower-density population and 13.4 % for higher-density population. Presented result show that reliable sampling of low density populations require enormous sampling intensity, which cannot be accomplished by manual mapping, and may be challenging even for machine vision systems and other automated methods.

## **New methods of maize cultivation with weed strips: Impact assessment on indicators for biodiversity**

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There has been a strong increase in conventional cultivation of maize in Germany since energy policy 2007. High yields are achieved, however, targets of biodiversity are mostly not included. Therefore, new methods of maize cultivation have been developed while retaining standard maize sowing technology, however with reduced tillage and a reduction in using pesticides. Under this method of micro segregation production stripes for maize as well as habitat stripes for wild herbs are itemized established. In 2016/2017 conventional as well as ecological maize cultivation methods upgraded by micro segregation were reviewed and assessed in trial areas (8 x 6 m, of each variant n = 4). Therefore vegetation structures as well as agrotechnical dates were recorded during breeding season of Skylark (*Alauda arvensis*), indicator species for biodiversity. Habitat assessment of cultivation variants were made by taking into consideration landscape data with data given of vegetation structure as well as the abundances of Skylark. Using the method »Moving Window Growth« for rate of growth and »Moving Window Abundance« for abundance course valuation parameters were derived for the single test versions. The results show that under conventional cultivation conditions there is a low habitat impact for the Skylark in maize fields. These areas form a sink biotope with successive reduction of Skylark population over the years. All variants with environmental upgrading lead to considerably better habitat conditions of maize cultivation areas. A key factor for these advantages is the established weed flora of approx. 33 to 47 % of maize area and the effects of partial soil management and the reduced application of herbicides amounting to only 53 to 67 % of the area. This clearly shows the function of wild herbs and their establishment, here in form of weed stripes, for positive effects on biodiversity in the maize cultivation.

## **Weeds and other flowering wild plants as important food source for pollinators in orchards**

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Biodiversity of invertebrates in orchards is strongly influenced by the occurrence of flowering plants. The flowering period of fruit trees is relatively short. One way how to support the occurrence of pollinators and other beneficial species related to nectar and pollen availability is to establish flowering stripes by sowing mixtures of crops with long flowering period. But there are also weeds and other wild plants, that occur and flower during the whole vegetation period for free.

We monitored flowering period of 10 fruit tree species and 128 species of dicot weeds and wild plants once a week during the vegetation period starting when all-day frosts were over and ending in autumn at the beginning of the first frost period. Monitoring was done in orchards at the Experimental field of the Faculty of Agrobiolgy, Food and Natural Resources in Prague - Suchdol in 2014, calendar week numbers were used.

Flowering of fruit trees started in 10<sup>th</sup> week (*Cornus mas*) and ended in 19<sup>th</sup> week (*Malus domestica*). Highest number of fruit species were flowering during 14<sup>th</sup> to 16<sup>th</sup> week (7 – 9 species). Weeds and wild plants started to flower 3 weeks earlier (*Veronica polita*, *Veronica persica*, *Stellaria media*, *Bellis perennis*) and continue to flower until the end of monitoring period (week no. 39). The highest numbers of flowering species were observed between 22<sup>nd</sup> and 31<sup>st</sup> week (80 – 104 species).

Weeds and wild plants in orchards start to flower earlier than the fruit trees and continue in flowering all through the vegetation period. When inter-row spaces and neighbouring areas of the orchard are properly managed, they can provide many flowering species as a food source for beneficial insects.



### **Pollinator attractiveness of five weeds in cereal systems**

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Pollinators in agro-ecosystems worldwide have declined alarmingly in recent decades due to poor agricultural practices such as intensive use of pesticides and monocultures. There was also a decline due to the reduction of wild areas where pollinators can feed and shelter. Weeds in agroecosystems may help maintain biodiversity by attracting pollinating insects.

In order to study the attractiveness of some native weeds to pollinators, a two-year trial was set up at Viladecans (Catalonia, Spain) in 2.5x2.5 m plots with 3 repetitions. Attractiveness of five different species (*Sonchus oleraceus*, *Papaver rhoeas*, *Daucus carota*, *Malva sylvestris* and *Convolvulus arvensis*) and a combination of all five in equal percentages was studied. Sampling was carried out with visual observations of insect visits to flowers in each plot, twice a week for 5 minutes in the morning. The observed insects were grouped into seven functional groups: bees, beetles, butterflies and moths, hoverflies, true bugs, wasps and other insects.

There were significant differences between the weeds at the level of attractiveness. *P. rhoeas* and *D. carota* were the weeds that showed the greatest attractiveness to pollinators, although *P. rhoeas* attracted mainly bees and beetles, while *D. carota* attracted bees, beetles, hoverflies and true bugs. The mixture of weeds attracted significant amounts of bees, beetles and true bugs and *C. arvensis* attracted predominantly bees and beetles. *M. sylvestris* and *S. oleraceus* were the species that showed an overall lower attractiveness for pollinators, the later probably due to the fact that its flowers were only open for a very short period each day.

We can conclude that *P. rhoeas* and *D. carota* were the best species for attracting pollinators because they attracted the greatest proportion of bees, which are important pollinators of crops. *D. carota* also attracted the greatest proportion of hoverflies, which are important predators of pests.

## Impact of flowering plants in a cereal crop field-margin on pollinators and predatory mites

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Intensive farming and monocultures have been blamed for the decline of habitats for plants and associated arthropods. Our aim was to evaluate the capability of a selected plant mixture, sown along the margin of a cereal crop in an intensively cultivated area in attracting pollinating insects and to measure its impact on predatory mites. The experiment was established in a 2ha cereal field in Boeotia, Greece. Plant species were selected from several families, excluding potential weeds and alien species. The seeds originated from native populations and were sown with a seed-drill in autumn 2015 along the field margin, in a 2m\*200m strip. An area of equal size in a conventional field margin, served as control. Three biweekly measurements in five 14m<sup>2</sup> plots designated in the sown and control strips, included flowering/species (% of surface cover) and attracted Hymenoptera pollinators (counts of insect-visits on flowers for 4'). Vegetation samples were collected from the strips and the crop for mites' extraction. Sown species that reached flowering were *Trigonella foenum-graecum* L., *Lathyrus clymenum* L., *Glebionis coronaria* (L.) Cass. ex Spach, *Coriandrum sativum* L., and *Trifolium resupinatum* L. The mixture, which flowered from mid-April to late May, had significantly higher percentage of flower-cover compared to the control and attracted Apidae (honey bees and bumble bees) and mining bees. Main flowering species in the control was *Sinapis arvensis* L. which attracted mining bees. The sown mixture and the crop had significantly more predatory mites compared to the control, probably due to the presence of their prey in these sites. Our results suggest that field margins with limited vegetation in intensively cultivated areas, can be enhanced with selected plant species to support hymenoptera pollinators and function as a »predatory mite bank« with potential benefits for the crop.

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## **Location, climate and weed distribution influence sheep carcass damage by weed seed in Australia**

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Weed seed contamination of sheep carcasses and pelts is of critical importance in New Zealand and Australia, two countries which account for more than 80% of the global sheep meat trade. Seed contamination frequently increases production costs, hinders livestock welfare and threatens quality of meat products. *Hordeum* spp. (barley grass) and *Bromus* spp. (brome grass) are annual Australian weeds that are native to the Mediterranean and specifically associated with seed contamination in sheep. In recent years, the distribution of both species has increased across southern Australia, potentially due to herbicide resistant populations and adaptation to diverse climatic conditions. Recent anecdotal evidence indicates increased weed carcass damage within Australian abattoirs, a trend potentially associated with weed distribution patterns. An understanding of the current prevalence of seed contamination across Australian states and the factors associated with incidence is imperative for effective mitigation and subsequent maintenance of quality standards in sheep meat exports. Analysis of Australian abattoir datasets combined with examination of regional climatic records were undertaken using linear mixed models to evaluate the factors influencing carcass damage across southern Australia. The spatial distribution of carcass contamination, *Hordeum* spp. and *Bromus* spp. across Australia were also spatially characterised. Results indicated seed contamination was significantly associated with state, region, animal age, sex and abattoir. Clear relationships also existed between distribution patterns of carcass contamination and prevalence of *Hordeum* spp. and *Bromus* spp. Mean monthly rainfall and elevation were also noted as significant climate factors contributing to contamination. In addition, complex interactions were noted between mean monthly temperature and state, and between elevation and date. Results highlight the need for further research regarding integrated management of key annual grass weeds contributing to seed contamination across Australia.

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## **The genetic diversity of wild and cultivated cornflower populations: implications for conservation**

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INRA, DIJON, France

The cornflower, *Cyanus segetum* Hill, 1762 (formerly *Centaurea cyanus* L.) is an iconic arable plant that was historically widespread in Western Europe, but has shown a marked demographic decline since the mid-20<sup>th</sup> century. The species is distinctively attractive due to its large blue-coloured flowers and is an important source of nectar for pollinating insects. For these reasons, populations from horticultural or cultivated origins are frequently sown within flower fallows and flower strips. However, cultivated populations may be of non-local origin and have a reduced and/or divergent genetic variation. Therefore, replacement of local populations by cultivated ones or gene flow from cultivated populations to wild populations may represent a threat for the in situ conservation of wild cornflower.

In this study, we described and compared the genetic diversity and genetic structure of wild populations sampled throughout France with that of plants sampled in flower fallows and from commercial seed lots. Plants originating from more than fifty wild populations, seven flower fallows and twelve commercial seed lots were genotyped with seven microsatellite markers.

A large genetic diversity and relatively low genetic differentiation ( $F_{st} = 0.128$ ) was revealed among wild populations, indicating a rather low genetic impact of demographic regression and population fragmentation on cornflower. Most horticultural populations had a different genetic makeup than wild populations. Flower fallows were genetically intermediate, suggesting that genetic mixing between wild and horticultural sources takes place within them. As this may negatively impact the local adaptation of wild populations, we advocate that cornflower seeds of wild, local origin are used in seed mixtures for flower fallows and flower strips.

## **The influence of climate conditions on weed species diversity under semi-arid conditions**

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**Background and objectives:** climate change studies have predicted a general change in seasonal temperature and rainfall patterns, but there are high levels of uncertainty about the nature of local changes. One aspect scarcely explored of climate change is the possible impact on the geographic distribution of agricultural weed species. This aspect is crucial in the Mediterranean regions, and the impact of these changes also are dependent of farm management techniques.

In the semi-arid regions of central Spain, under rainfed conditions, cereal crops are most prevalent. In these cereal agro-ecosystems, conservation agriculture (CA), which includes direct sowing and minimum tillage, is very commonly employed. However, considering the general trend towards an earlier spring, there is a lack of information about the presence of weed species and variations with respect to climate conditions and tillage system.

The general objective of this project is to study the influence of mean temperatures on the diversity of weeds present in semi-arid cereal agro-ecosystems in Spain.

**Methods:** Information about the distribution of weed species in cereal agro-ecosystems susceptible to temperature was obtained from different data sources recorded over the last 10 years.

**Results:** A map was created to visualise the distribution of weed species and temperatures over the last 10 years. A classification list of weed species was created to highlight effects of conservational agricultural practices under different climatic conditions.

**Conclusions:** In order to create effective site-specific weed management programs, the effects of climatic conditions and agronomic practices must be taken into account.

## Long-Term Winter Wheat Cropping Influence on Weed Seedbanks

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Winter wheat is one of the most important crops on the Balkans. To access the wheat-based cropping effect on weed seedbanks, data were used from a long-term experiment »Crop rotation« located on experimental field of the Institute of Field and Vegetable Crops in Novi Sad. The selected study treatments were as follows: wheat monocultures, 2-year crop rotation (wheat-corn) and 3-year crop rotation (wheat-corn-soybean). In order to determine the weed seedbank, soil samples were taken in the autumn from three depths: 0-15 cm, 15-30 cm and 30-40 cm. By the method of physical extraction, the presence of weed species in the weed seedbank is established, while the seedling emergence method showed the species whose seeds had passed the state of dormancy and were able to germinate in the next period. Seeds were extracted from the soil by washing and they were determined and counted. The obtained results showed that in all three wheat cultivation systems and three depths, the number of species is similar (9-11), only in the 3-year rotation in the under plow depth 7 species were found. There are differences in species diversity as well as in the number of present seeds. It is estimated that in the plow layer of monoculture wheat there are about 281250 seeds m<sup>-2</sup>, 2-year crop rotation of 102750 seeds m<sup>-2</sup>, while in the rotation of wheat with maize and soya the smallest number is shown (75000 seeds m<sup>-2</sup>). By the seedling emergence method in controlled conditions of greenhouses, the best germination in the monoculture and the 2-year crop rotation had the species *Papaver rhoeas* L., *Bilderdykia convolvulus* L., *Chenopodium hybridum* L., *Consolida regalis* S.F.Gray, while in the samples of 3-year crop rotation seedling of *Anagalis arvensis* L. and *Sorghum halepense* L. appeared.

**Maize response to weed interference under changed production technology**

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Weeds interfere with the utilization of resources and thus adversely affect <https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/crop-production>. Intensity of their interference is especially important during stages when yield is being determined. Weed species differently respond to herbicides and this may reflect differences in selection pressure caused by a cropping system. Under changed row space of maize and plant density, weed abundance and biomass production are also different. Fertilizer application and soil fertility have a definite influence on weed diversity and growth, and maize leaf area index, harvest index and yield.

The investigations were conducted in the MRI Zemun Polje, Belgrade, Serbia during 2014-2016. Nitrogen fertilizer was applied in two forms: standard urea and slow release urea with urease inhibitor, applied at maize stage BBCH 05. Herbicides mix for grasses and broadleaf weeds were applied pre-emergence (s-metolachlor + mesotrione) and post-emergence (nicosulfuron + mesotrione). Maize was sown with row space of 50 cm and 70 cm - 59.500 and 83.333 plants per ha, respectively. Three weeks after herbicide application, dry matter was measured from uprooted weeds from 1 m<sup>2</sup> randomly selected in each elementary plot. Maize parameters – LAI, HI and grain yield were evaluated when plants fully matured. The data were processed by ANOVA.

Weed dry matter was highly dependent on the year and time of herbicide application, as well as of some interactions: year x urea form, year x herbicide application and year x urea form x herbicide application. Differences in average weed dry matter between urea forms and maize row space were not noticeable. The lowest weed dry matter was obtained after post-emergence herbicide application (5.01 g m<sup>-2</sup>). Based on the correlation analysis, weeds very significantly and negatively influence leaf area index ( $R^2 = -0.524$ ), harvest index ( $R^2 = -0.598$ ) and grain yield of maize ( $R^2 = -0.688$ ).

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## **Beyond species richness: disentangling contributions of cover crop traits to weed suppression in mixtures**

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Ecological theory suggests that diverse plant communities should be more resistant to plant invasion; however, empirical studies with cover crops often fail to show greater weed suppression in species-rich mixtures compared to that observed in the most weed-suppressive cover crop grown in monoculture. Our objective was to examine how the traits of cover crop species grown in mixtures influence weed suppression and to develop new metrics for assessing the relative performance of mixtures with regard to weed suppression and effects on subsequent cash crops. We conducted a two-year field experiment in southeast New Hampshire, USA. Treatments were mixtures and monocultures of annual broadleaf, legume, and grass cover crops aimed at filling three distinct temporal niches during the growing season: early-, mid-, and late-season. Cover crop and weed biomass were sampled prior to incorporation of cover crop residues. Following incorporation of residues, an indicator crop was sown to measure the effects of the previous cover crop treatment on subsequent crop performance (yield) and weed suppressiveness. We calculated the land equivalent ratio (LER) and several other synthetic indices to quantify the performance of cover crop mixtures and their effects on a subsequent test crop. For each group of cover crop treatments, cover crop mixtures tended to result in over-yielding ( $LER > 1$ ), but did not suppress weeds better than the most weed suppressive cover crop grown in monoculture. Indices that accounted for individual cover crop species traits were better predictors of weed suppression and performance of a subsequent test crop than was cover crop biomass alone. Our results suggest that species trait information can be used to create cover crop mixtures that effectively enhance multiple ecosystem services, including weed suppression.



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**Inventory of the biofunctional weeds present in sugar cane in Costa Rica**Angela Soto Saenz

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Several weeds associated with sugarcane plantations have the potential to host beneficial insects, such as predators and parasitoids, which can provide multiple ecological services, including pest control. In this study, the diversity of weeds was quantified in the six contrasting sugarcane regions of Costa Rica in 2017, to accomplish it we identified each plant taxonomically and performed a similarity analysis with the Jaccard index for each region. There was a total of 244 species distributed among all the regions, represented in 51 families. The Turrialba (east) and San Carlos (north) regions accounted for the highest diversity of weeds, and the region of San Carlos had the highest number of plants reported to host natural enemies of pests. The next phase of this study will study morphological traits of these plants and relate them with their potential to provide food, shelter and alternative prey to natural enemies of pests. This information will guide future vegetation and insect management strategies in sugarcane plantations in order to maximize plant ecological services and minimize chemical pest control.

### **Weed seed-bank in short-term and long-term grasslands**

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In old grasslands, perennial weeds like *Taraxacum* spp., *Ranunculus* spp. and *Elymus repens* may become frequent. There is scarce information on how well weed seeds generally survive in long-term grasslands. If there is less survival of weed seeds in long-term grassland the renovation in old swards with ploughing and reseeding can result in less weed seedlings and less need for chemical weed control in the seeding year. The aim of this study was to determine the weed seed-bank in swards of various age. Two long-term grassland experiments in south-western Norway (58°N and 61°N) that included treatments with ploughing and reseeding every 3<sup>rd</sup> or 6<sup>th</sup> year, or permanent grassland older than 20 or 40 years, were used to determinate weed seed-bank. Soil samples from 0-5 and 5-18 cm soil depth were collected in spring 2016. Weed seeds were estimated with a procedure consisting of three germination periods in the greenhouse interrupted by dry or cool conditions during an entire one-year period. Overall, we detected more seeds per volume soil in the upper soil layer than in the lower soil layer. In one of the experimental fields, the total abundance of weed seed-bank was less in long-term grasslands compared to grasslands that have been renewed every 3<sup>rd</sup> or 6<sup>th</sup> year. In contrast, the age of grassland had no effect of weed seed-bank at the second location. We suppose that there, probably, was some seed supply and transport of seeds down into the soil even if the sward was not ploughed for several decades. Another plausible explanation is that the seeds of particular species may survive in the soil for several decades.

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### **Will biodiversity regained in wheat fields?**

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Wheat is one of the most important staple foods across all parts of the world, covering large acreages. Studies on weed flora in wheat fields as well as other crops in Europe have showed biodiversity loss due to changing farming practices such as fertilization and herbicide application since the World War II. It is reported that recent attempts to regain diversity are not as successful as expected, although promising in the longer run. Turkey, where less input consumption and historically starting late for use of modern inputs comparing to Western world has less biodiversity loss as it was learnt from weed surveys which go back to 1944. Although neither field surveys nor seed controls have been conducted regularly, more of the weed surveys were conducted after the 1960s. Loss of biodiversity was varied depending on the region. It is predicted that the loss is higher in South and West regions than North and East regions of Turkey depend on the consumption of modern inputs and cropping system. According to an evaluation from the South East Anatolia Region in five decades; the reduction of species is about 2/3<sup>rd</sup>, the species widespread over 50% of fields declined significantly and dense over 1 plant/m<sup>2</sup> in the past such as *Lactyrus erectus* and *Tordylum apulum* were not seen the further studies. It is concluded that developing and less developed countries have chance to conserve even regain biodiversity if actions to conserve biodiversity taken immediately with the lesson learnt from the experience from developed countries. The factors affecting the biodiversity loss and some actions mainly change the agricultural practice or system will present.

### **Micro-segregation in maize cropping- a chance for farmland biodiversity?**

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The rapid loss of farmland biodiversity has provoked a discussion on whether nature conservation should be integrated into normal field production or separated into designated areas. With the micro-segregation approach we want to combine both on the same field: strips with optimal condition for crops and strips with optimized for biodiversity. Today maize is the most promising crop for this approach in Germany: it provides enough space between the crop rows. Properly managed, this space could be used to establish habitat strips for flora and fauna without reducing the yield substantially. Therefore a mixture of wild plants is sown to establish the habitat strips after harvesting the preceding crop in the previous year. Strip-till techniques enables maize-sowing in between the flowering habitat stripes. Band-spraying spares the habitat strips, protects the plants against the herbicides and reduces herbicide application. In this way the habitat strips stay undisturbed for over one year. A key criterion for the success of this approach is the site specific mixture of sown wild plants. On the one hand they must be competitive to voluntary weeds. On the other hand they must form a habitat strip which is rich for biodiversity and secure the maize yield.

## Weed species and vegetation analysis of the selected vineyards in Moravia

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The goal of this work was to create a list of plants growing on the selected vineyards tracks in Moravia region and to evaluate significance of present plant species on vine growing. Four winery villages from Moravia winery region were chosen: Mikulov (11 vineyards tracks), Strážnice (10 vineyards tracks), Velké Pavlovice (7 vineyards tracks) and Znojmo (5 vineyards tracks). In these villages, the areas with vineyards were located at first. Then monitoring of vineyard vegetation was carried out between June and September 2016 and 2017. Evaluation of vegetation was made using a floristic list of the found species. In selected area of vineyards tracks, the paths through vineyard were determined. During passing through, the plant species were recorded. In total, 299 plant species was recorded. Some of the recorded plant species can be considered as competition for grapevine because of similar environmental needs. The most dangerous were the weed species with deep roots and the species of tall growth (*Cirsium arvense*, *Rosa canina*, *Robinia pseudacacia*). Also, the weed species (*Ailanthus altissima*, *Amaranthus albus*, *Arrhenatherum elatius*, *Calamagrostis epigejos*, *Conyza canadensis*, *Epilobium ciliatum*, *Erigeron annuus*, *Lycium barbarum*, *Oxalis fontana*, *Portulaca oleracea*, *Robinia pseudacacia*, *Solidago canadensis* and others) were recorded. These species are invasive or expansive and tend to spread in Czech Republic. The variety and diversity of vineyard vegetation provides the basis for the richness and stability of the entire vineyard ecosystem. Vineyards can be with proper management area where a space for rare plant species can be created and they can become a source of biodiversity. Nevertheless, vineyards are area where invasive and expansive plant species occur. This work was supported by project DG16P02R017 »Viticulture and winery for preservation and restoration of cultural identity of winery regions in Moravia «.

## **Competitive ability and spatial aggregation of weed species in organic cereal fields in Spain**

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Spatial aggregation has been suggested as one of the mechanism permitting the coexistence of plant species. This mechanism could also explain the maintenance of diverse competitive strategies in weed communities. In that case, spatial aggregation of conspecific individuals would benefit weaker competitor weed species, whereas stronger competitors would be disadvantaged. Moreover, stronger competitors would show greater dispersal than weaker competitor species. Therefore, traits determining spatial aggregation patterns and those related to competitive ability should be related.

Here, we address whether the growth of weed species is determined by the identity of neighbors (conspecifics vs. heterospecifics) and the degree of conspecific aggregation (DCA). To do that, we sampled weed species in 12 organic winter wheat fields in Andalusia (Spain) during 2016-2017 season. We recorded weed abundance, cover and height in ten 1 m<sup>2</sup> plots per field.

A total of 128 weed species were registered. We selected 20 species to perform the analyses according to their frequency and abundance in the fields. For each species and in each plot, we computed the following variables: a) the average biovolume as a proxy of its growth; b) the probability to occur with conspecifics as the ratio between the number of individuals of the focal species to the total number of individuals in each plot (identity) and c) DCA as identity minus the frequency of occurrence of the focal species in the field. Linear mixed models were performed with biovolume as the response variable, and identity, DCA, and the total number of individuals per plot as fixed factors. Species nested in fields was included as a random factor. A three-way significant interaction would indicate that DCA influenced the difference in the species competitive response to conspecific versus heterospecific neighbors.

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## **Weed host of root-knot nematodes (*Meloidogyne* spp.) found in vegetable growing areas in Turkey**

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Root nematodes are endoparasitic species that have close to 100 species and can be feed on almost all plants on the earth. Relationships between root-knot nematodes and weeds can be assessed in two basic contexts: firstly they can be host to the root nematodes; while the other is that weed and their some products can be used for the control of root-knot nematodes.

In this study, the identified species that host to the root-knot nematodes in vegetables grown in Turkey are given and reviewed together.

In the study conducted in Central Anatolia in the 1980s, while talking about the host of *Portulaca oleraceae* and *Solanum nigrum*, in 2000 it was again *S. nigrum* which emphasized the high level of host to *M. javanica* in Adana.

It has been determined that there were 17 different species of weeds from 9 families including Amaranthaceae, Asteraceae, Chenopodiaceae, Cyperaceae, Fabaceae, Malvaceae, Poaceae, Portulacaceae, Solanaceae, in vegetable fields in the Çukurova region are infested with different species of root nematodes before.

For Black Sea Region, in our studies conducted in vegetable growing areas including protected greenhouses, *P. oleraceae*, *Datura strumarium* and *Xanthium* sp. were the most founding weed hosts for *Meloidogyne* spp.

It is very important to know that which weed species could be the host for root- knot nematodes especially in infested vegetable growing areas, because these weeds would be able to the alternative host for the cash crop and control strategies must be considered this.

**S13-O Biologically Inspired Weed  
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## Impact of *Brassica juncea* biofumigation on viability of propagules of pernicious weed species

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Biofumigation may be a promising tool for depletion of persistent weed seed/bud banks. This technique is based on the incorporation of glucosinolate-rich fresh chopped plant biomass into the soil, which releases isothiocyanates with herbicidal properties. To gain acceptance by farmers and foster its implementation, the biofumigation process should be optimised. This study aims at (1) determining sensitivity of economically important and hard to control weed species to *B. juncea* biofumigation; (2) elucidating pedo-hydrological factors affecting efficacy of *B. juncea* biofumigation. To determine interspecific differences in susceptibility to biofumigation, seeds and vegetative propagules of 15 weed species were buried at three soil depths (5, 10, 20 cm) and subjected to three dosages of fresh, finely chopped *B. juncea* tissue (0, 70, 200 ton ha<sup>-1</sup> uniformly incorporated to 10 cm depth), in presence or absence of a plastic soil cover. To assess the impact of pedo-hydrological factors, seeds of 10 species were buried in 20 L pots with equal amount of *B. juncea* biomass but different soil organic matter content, soil moisture content and soil temperature. Propagules were exhumed 14 days after burial and were tested for viability by a germination test followed by a tetrazolium test.

In general, efficacy of biofumigation increased with decreasing seed mass and burial depth, and increasing dosage of *B. juncea* tissue. Biofumigation was highly effective (mortality >85%) against small-seeded species, moderately effective (mortality >50%) against annual grasses and poorly effective (mortality 0-20%) against hard-seeded and large-seeded species. Mortality of vegetative propagules was high (>90%) for *Sonchus arvensis*, *Equisetum arvense* and *Calystegia sepium*, medium ( $\pm 60\%$ ) for *Elytrigia repens* and *Polygonum amphibium*, and low ( $\approx 0\%$ ) for *Cyperus esculentus*. Viability reducing capacity of *B. juncea* biofumigation was most pronounced in moist warm soils and in the presence of a plastic soil cover. Impact of soil organic matter content was less clear-cut.

## **Allelopathy and interaction of a momilactone deficient rice mutant with barnyardgrass**

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Many laboratories and field screening experiments have shown that rice is allelopathic and secretes allelochemicals into its surrounding environments. A number of compounds, such as phenolic acids, fatty acids, phenylalkanoic acids, hydroxamic acids, terpenes and indoles, have been identified as potential allelochemicals of rice (*Oryza sativa* L.). However, momilactone B of them is playing a particularly critical role in rice allelopathy. Rice plants secrete momilactone B from their roots into the neighboring environments over their entire life cycle at phytotoxic levels, and momilactone B seems to account for the majority of the observed rice allelopathy. A momilactone deficient rice mutant contained equal amount of phenolic acids to its wild type but lost allelopathic activity. When barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) and rice seedlings were grown together with enough nutrient conditions, allelopathic activity and momilactone secretion level of the rice increased. Barnyardgrass root exudates also increased rice allelopathy and the levels of momilactone B production and secretion from the rice. Momilactone B possesses strong growth inhibitory activity against barnyardgrass. Thus, barnyardgrass-induced rice allelopathy may be due to the increased momilactone B production and secretion. However, the momilactone deficient rice mutant did not increase the allelopathic activity. Rice may be aware of the presence of neighboring barnyardgrass by detection of the key compound in barnyardgrass root exudates, and this sensorial function may trigger a signal cascade resulting in increasing rice allelopathy through increasing production of momilactone B in rice and secretion of momilactone B into the surrounding environments.

**Effect of different mulches and bio-herbicides on weed flora in organic vineyards**

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The most frequent weed management practice in organic vineyards in Raimat (Lleida, Spain) is mechanical cultivation within vineyards rows, but these practices cause damages in young vineyards and affect the soil quality by increasing erosion. With the objective to evaluate alternative weed management strategies, two different trials were carried out in 2017. In the first trial seven treatments were established in a randomized complete block design. Treatments were replicated three times and included: 1) mechanical cultivation (in-row tiller); 2) two bio-herbicides (concentrated vinegar 20% at 312L/ha and a mix of fulvic acid and humic acid at 538L/ha); 3) three different mulches of barley, alfalfa and Festuca arundinacea straw, and another composed of pine leaves and bark. In the second trial, a similar design was established considering the same four mulches at two different thicknesses: 5 and 10cm. In both trials mulches were established in March 2017. Cultivation was carried out in May, July and September according to the presence of weed cover. In trial 1 the application of both bio-herbicides was made at three different dates according with the emergence of weed seedlings (May, June and July). In both trials regular estimation of weed abundance (cover percentage) was estimated for each treatment in three different 3m x 0.8m plots. Throughout the whole season, the average weed cover observed in cultivation was 10.8% with no differences observed for bio-herbicide values (9.8% and 8.1%). The weed cover estimated in the different mulches was significantly lower, with values of 3.9% (pine), 2.1% (F. arundinacea), 0.7% (alfalfa) and 0.7% (barley). Barley, alfalfa and pine mulches at 10cm were more effective in reducing weed cover than those at 5cm, with significant differences from mechanical cultivation values.

## Effect Of Some Organic Herbicides On Weeds In Sunflower Fields

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Successful weed control should start early in the season to enhance the crop growth and competitiveness. In most studies, interspecific competition between weeds and crop plants occurs in the first four weeks after crop emergence and therefore, it's ideal to control weeds during this period. In 2015, a field experiment was conducted in the Adana province, Turkey, to investigate the effectiveness of three organic herbicides on weeds infesting sunflower-growing fields as an alternative to synthetic herbicides. The experiment was laid out in a randomized complete block design (RCBD) with four replications. In this experiment, plot size was 2.1 x 1.75 m (3.68 m<sup>2</sup>). The organic herbicides used were corn gluten meal (4 tone/ha), clove-cinnamon oil (100 l/ha) and amino acid salt (100 l/ha). All herbicides were incorporated into the soil before planting, then the emerged weed species were regularly recorded. After four weeks, the emerged weeds were harvested and the fresh weights were recorded. Dry weights were also determined after plants were dried for 48 hours in a forced-air oven at 105°C. As a result, significant differences in the emerging weed composition were observed among the different treatments. A total of 12, 8, 6 and 6 weed species were identified in the control, corn gluten meal, amino acid salt and clove+cinnamon oil treatments respectively. Common lambsquarters (*Chenopodium album* L.), redroot pigweed (*Amaranthus retroflexus* L.) and purple nutsedge (*Cyperus rotundus* L.) were the most abundant species in the study area. Generally, organic herbicides were effectively reduced the weed population density. Across the examined organic herbicides, the highest level of biomass reduction was observed with corn gluten meal and amino acid salt applications. It was concluded that these organic herbicides could be an alternative option to the synthetic herbicides.

**Bioherbicide activity of allelopathic compounds released from *Cytisus scoparius***

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*Cytisus scoparius* (L.) Link, a shrub species native to the Atlantic region, is considered as an undesirable weed and highly invasive species in many parts of the world. Part of its invasive nature is due to its allelopathic potential. In previous studies from our lab, *C. scoparius* biomass was appraised as a bioherbicide green manure with promising results, and the chemical profile of flowering aerial parts was elucidated by HPLC (for phenolic compounds) and GC+GC-MS (for volatile organic compounds–VOCs). After studying the phytotoxic effect exerted by each isolated compound in the phenolic (water-soluble) or volatile profile, we raised the question ‘which fraction is the responsible for the phytotoxicity observed: the phenolic, the volatile fraction, or the interaction of both?’ In the present work, mixtures of phenolic compounds and VOCs were prepared by mimicking the concentrations and proportions of the natural composition of *C. scoparius*. Then, we explored the *in vitro* phytotoxicity of the phenolic and the volatile fractions, separately and concomitantly, on the germination of *Amaranthus retroflexus*. Both volatile and phenolic fractions significantly inhibited the germination of *A. retroflexus* with respect to the control ( $P \leq 0.001$ ), VOCs being the most phytotoxic. Although both fractions acting together produced inhibition of total germination up to 90%, no statistically significant interaction between the volatile and the phenolic fractions was found. Then, compounds released from *C. scoparius* biomass used as green manure are argued to have an additive effect, but not synergistic.

## **Amino acid overproduction confers resistance to plants against *Phelipanche* and *Orobanche* spp.**

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*Orobanche* and *Phelipanche*, commonly known as broomrapes, are dicotyledonous holoparasitic flowering plants that cause heavy economic losses in a wide variety of plant species. The sensitivity of broomrapes in various developmental stages to exogenously applied amino acids was previously reported. Because of the direct anatomical-physiological connection between the parasite and its host, one can hardly distinguish direct influence of exogenous amino acids from indirect influence caused through damaging the host plant. Therefore, in order to evaluate the toxicity of exogenous amino acids to these parasites, we used tissue culture of *P. aegyptiaca* and *O. cumana*. The tissue cultures were grown on media containing various amino acids, at 1 - 5 mM. Callus growth and biomass accumulation were evaluated. The amino acids phenylalanine (Phe) and threonine (Thr) completely inhibited *P. aegyptiaca* calluses growth at  $\geq 1$  mM. Arginine (Arg), tryptophan (Trp), tyrosine (Tyr) and methionine (Met) were active at  $\geq 2$  mM. *O. cumana* callii were less sensitive. Phe inhibited their growth at  $\geq 3$  mM, Tyr and Lysine (Lys) at  $\geq 4$  mM. Exploring the ability of transgenic tobacco plants, with higher levels of various amino acids, to inhibit the growth of *P. aegyptiaca* in greenhouse experiments revealed that overproducing Thr, Met or aromatic amino acids provides a better resistance to the parasite compared to wild type. The resistance correlated with the amino acid concentration in the tobacco roots.

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## **Improving effectiveness of natural herbicides by environmentally friendly and sustainable nanoformulation**

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Natural compounds and living organisms have still a limited use as natural and safe herbicides, and only a few reached the market, despite their attractiveness and the efforts made in research. Very often those »products« have negative characteristics compared to the traditional herbicides, e.g. higher costs, lower effectiveness, lack of persistence, inability to reach and penetrate the target plant. On the other side, nanotechnologies are having an enormous impact in all the human activities, including agriculture, even if often they are not environmentally friendly and could even have adverse effects in the agriculture and in the environment. Thus, some suitable nanotechnologies could facilitate the development of nano-natural herbicides, more effective and more environmentally friendly, with improved efficacy, minimized dose use, increased shelf-life and persistence, able to recognize the target, having a formulation facilitating its release. The present communication aims at overview the potential of safe and environmentally friendly nanoformulations to be suitable for natural herbicide development. A specific example will be given, i.e. the use of inuloxin formulated by cyclodextrins. These latter are a group of structurally related cyclic oligosaccharides formed during bacterial digestion of cellulose, consisting of ( $\alpha$ -1,4)-linked  $\alpha$ -D-glucopyranose units and containing a somewhat lipophilic central cavity and a hydrophilic outer surface. They are considered GRAS compounds, and some are used for pharmaceutical products, food products, cosmetics and other commercial products mainly as solubilizing agents to increase water-solubility of lipophilic compounds, to increase both chemical and physical stability of various compounds, or to slower their bio-availability. Inuloxin is a natural sesquiterpene lactone isolated from the aerial parts of *Inula viscosa* (family Asteraceae), a widespread Mediterranean plant, that proved to have some herbicidal properties, particularly against parasitic weeds. The first information on inclusiveness properties of cyclodextrins, stability, effectiveness and bio-availability of the nanoformulated product will be presented.

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## **Inhibitory effect of *Origanum* species hydrosol by in vitro assays on weeds and bioindicators**

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Oregano is considered one of the most important temperate culinary herbs. Greek oregano (*Origanum vulgare* ssp. *hirtum*) and Turkish oregano (*Origanum onites*) are the most widespread in Greece with their hybrid, *Origanum x intercedens* occurring in lower populations. Several experimental strategies were carried out to assess the potential phytotoxic effects of oregano plant extracts on *Avena sativa*, *Lemna minor* and *Lolium rigidum* investigating their inhibitory effects by *in vitro* assays. These species were cultivated in the Agricultural University of Athens using vegetative propagation material of native plants from the Greek island Ikaria in the Eastern Aegean. The plants were harvested at the full anthesis stage. According to the methodology, the flowering shoots were physically dried, their essential oil and hydrosol were extracted using hydrodistillation and finally, the extracts were analyzed using the gas chromatography-mass spectrometry (GC-MS). The chemotype analysis showed the high concentration of carvacrol, a main component in the essential oil, in the three studied *Origanum* species and particularly at the values 90,89%, 86,97% and 95,27 for *O.hirtum*, *O.onites* and *O.intercedens*, respectively. *In vitro* experiments were conducted in petri dishes, where the weed seeds and *Lemna* foliage were subjected to dose-response bioassays by the following concentrations of oregano species hydrosols, 100%, 50%, 25%, 12,5%, 6.25%, 3.12%, 1.56%, 0.78% and 0.39% v/v. The seed germination, the radical length and *Lemna* foliage growth were measured to evaluate the phytotoxicity of oregano species hydrosols. Estimating the Inhibitory Index ( $I_{50\%}$ ) based on dose-response curves, the data showed a great phytotoxic response, in descending order, *O. hirtum* hydrosol followed by *O. intercedens* and *O. onites*. Noticeably, *Origanum* hydrosols presented higher phytotoxic action compared to the phytotoxicity derived from the carvacrol bioassays and may have a potential herbicidal use.

### **Allelopathic potential of *Jatropha podagrica* Hook.**

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*Jatropha podagrica* Hook. (Euphorbiaceae) is an ornamental plant native to the tropical Americas and it is well known to have various biological properties. However, to date, the allelopathic properties of *J. podagrica* have not been reported. Therefore, the present research was conducted to investigate the allelopathic activity and to search allelopathic substances in *J. podagrica*. The leaf powder of *J. podagrica* was extracted with 70% aqueous methanol and cold methanol. Those extracts were combined and determined the growth inhibitory activity on cress, alfalfa, lettuce, foxtail fescue, timothy and barnyard grass. The extracts inhibited the growth of shoots and roots of all the test plants and the inhibition increased with increasing extract concentrations. The concentrations required for 50% inhibition ( $I_{50}$ ) of the leaf extracts on the shoots and roots of tested plants ranged from 0.05–13.20 mg D.W. equivalent extract/mL. The leaf extracts were then purified by several chromatographic steps. A growth inhibitory substance was finally isolated with HPLC and characterized as 6,7-dimethoxychromone by spectral analysis. 6,7-Dimethoxychromone significantly inhibited the growth of cress at concentrations greater than 0.3 mM.  $I_{50}$  values of 6,7-dimethoxychromone on the shoots and roots of cress were 1.0 and 0.8 mM, respectively. The present results suggest that 6,7-dimethoxychromone may contribute to the growth inhibitory effects of *J. podagrica* leaves. Therefore, the *J. podagrica* leaves and its bioactive substance could potentially be used as alternative options for control weeds. This research is the first report about the allelopathic activity and the presence of allelopathic active substance in *J. podagrica* leaves.

## **Artemisia arborescens and Hypericum empetrifolium: two pharmaceutical plants with potential herbicidal action**

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### *Artemisia arborescens* Vaill. and *Hypericum empetrifolium*

[https://www.google.gr/search?rlz=1C1ARAB\\_nGR499GR508&espv=2&biw=1280&bih=675&q=hypericum+empetrifolium+Willd&spell=1&sa=X&ei=s1MAVL\\_rOaXN7Aaaz4HYCw&ved=0CBcQvwUoAA](https://www.google.gr/search?rlz=1C1ARAB_nGR499GR508&espv=2&biw=1280&bih=675&q=hypericum+empetrifolium+Willd&spell=1&sa=X&ei=s1MAVL_rOaXN7Aaaz4HYCw&ved=0CBcQvwUoAA),

constrains of Mediterranean flora, have been used since ancient times for pharmaceutical reasons. This study aims to evaluate the phytotoxic activity of the aforementioned species. Leaves and inflorescences were submitted to hydrodistillation using Clevenger apparatus. Their essential oils were analysed by Gas Chromatography–Mass Spectrometry (GC-MS). The derived hydrosols were diluted at 100, 50, 25, 12.5, 6.75, 3.125, 1.56, 0.28, 0.14, and 0.07% v/v and were bioassayed for their inhibitory action using the bioindicators *Lemna minor* L. and *Avena sativa* L. as well as the weeds *Cyperus rotundus* L and *Lolium multiflorum* Lam. In petri dishes, the seeds germination and radicle growth of *Avena* and *Lolium*, the tubers vegetation of *Cyperus* and the foliage growth of *Lemna* in aqueous culture, were assessed for their response to the hydrosol application. The hydrosol's phytotoxicity was evaluated based on the inhibitory index ( $\text{Log EC}_{50}$ ) using the dose response curves (GraphpadPrism). *Hypericum* hydrosol showed the higher phytotoxicity in *Avena* bioassays compared to *Artemisia* hydrosol with values 0,0222 and 0,1432, respectively. Noticeably, *Artemisia* showed the higher phytotoxic effect compared to *Hypericum* hydrosol in *Lolium*, *Lemna* and *Cyperus* bioassays. Specifically, the values of inhibitory indices of *Artemisia* and *Hypericum* were respectively; in *Lolium* assays 0,7378 and 1,043, in *Lemna* 0,9841 and 1,190 and in *Cyperus* 1,116 and 1,528. Furthermore, the phytotoxicity of the main components of the tested hydrosols was also investigated. Camphor and borneol were identified in *Artemisia* and a pinene and a teprineol in *Hypericum*. In *Avena* bioassays, camphor showed the higher phytotoxicity followed by a pinene, a teprineol and borneol. Similarly, camphor showed higher phytotoxicity in *Lolium*, *Lemna* and *Cyperus* bioassays, followed by borneol, a pinene and a teprineol. Actually, the studied medicinal plants may be considered as a »phytochemical pool« with potential herbicidal use.

### **Crop selectivity and weed control efficacy of vinegar and pelargonic acid**

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Selectivity and weed control of vinegar and pelargonic acid were assessed in two greenhouse trials. One trial was carried out with vinegar on maize and the other with vinegar and pelargonic acid on rice. The study on maize was carried out in trays (20x30x5 cm) filled with soil with a history of maize cultivation maintained at the field capacity to permit weed emergence. Main weeds were: *Viola tricolor*, *Digitaria sanguinalis* and *Cyperus esculentus*. In each tray, six maize seeds were sown. Treatments based on one, two, three and four applications of vinegar (12% acetic acid at 500 L ha<sup>-1</sup>) were carried out at different timings: maize pre-emergence, emergence (BBCH 09), early post-emergence (BBCH 12-13), late post-emergence (BBCH 14-15). Twenty days after last treatment, weeds were counted and weed and maize biomass weighted. For the study on rice weeds, seeds of *Echinochloa crus-galli*, *Oryza sativa* (weedy rice), *Ammania coccinea* and *Heteranthera reniformis* were sown in pots and treated at 2-3 leaf stages (BBCH 12-13) with either vinegar (12% acetic acid) or pelargonic acid (18.8%) at the following rates: 10, 20, 30, 50, 70, 90, 100% of the label rate (pelargonic acid: 100 L ha<sup>-1</sup>; vinegar: 500 L ha<sup>-1</sup>). After 20 days weed biomass and visual efficacy were assessed. Maize biomass was not affected by treatment with vinegar at different growth stages. Best maize weed control was obtained with three treatments (pre-emergence+emergence+early post-emergence) with 96% and 98% weed density and biomass reduction, respectively. On rice weeds, pelargonic acid at 90% and 100% label rate always resulted in a higher weed control compared to vinegar at the highest rate.

**Fate of allelopathic compounds released from *Eucalyptus globulus* green manure**

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In the worldwide search for new strategies in sustainable weed management, the use of allelopathic plants incorporated into the soil as green manure can help control weeds by releasing allelochemicals into the environment. The measurement of when allelopathic compounds affect a plant, and when phytotoxicity peaks or diminishes is important to integrating allelopathy into residue management strategies. In our previous greenhouse experiments, *Eucalyptus globulus* Labill. leaves incorporated into the soil as a green manure showed a notable potential for weed control. But, 'what is exactly happening at chemical level?' and 'which are the compounds potentially responsible for the phytotoxic effects observed during greenhouse assays?' In the present study, *in-vitro* phytotoxicity bioassays and chemical analysis of eucalyptus leaves were carried out in order to explore the relationship between the temporal phytotoxic effects and the dynamics of chemical composition. For that, eucalyptus leaves were dug out from soil at different sampling times during 30 days and analysed for phenolic and volatile organic compounds (VOCs) by HPLC and HS-SPME/GC-MS, respectively. The phytotoxic potential of the aqueous extract (phenolic compounds) and the volatile fraction was tested on the germination and early growth of a model target species. *E. globulus* leaves incorporated into the soil as green manure showed a continuous release of different phenolic and volatile compounds during a 30-day period of decomposition. Both phenolic and volatile fraction had phytotoxic effects during the time assayed; however, the target process of phytotoxicity was different, phenolic compounds being the factor causing germination inhibition, and VOCs the responsible for growth reduction. The dynamics of release of this cocktail of allelochemicals into the soil environment may be the responsible for the phytotoxicity observed in both laboratory and greenhouse experiments.

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### **Biocontrol against the root parasitic plant species *Phelipanche ramosa*, branched broomrape of tobacco**

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The branched orobanche, *Phelipanche ramosa* L., is an obligate parasitic weed that has become, in France, a major agronomic problem in tobacco crops; yield and quality losses can be total and cause growers to reduce the producing area due to lack of available healthy plots or to abandon the crop. Because chemical management is not effective, biocontrol is certainly the alternative to propose but there is currently no biological control agent on the market to meet the expectations of producers and control this parasitic plant. Only a few microorganisms or molecules, are presented as potential and promising candidates in conclusion of scientific articles but the finalization is absent. A collection of 525 fungal isolates was made from 400 symptomatic orobanches taken from 28 plots representative of the tobacco growing regions in France to identify virulent fungi, adapted to the local pedoclimatic conditions and usable to control locally the development of orobanches. Indeed, pathogenicity tests, conducted in controlled bioassays and taxonomic identification based on morphometric and molecular criteria (ITS and EF1-alpha barcoding) are partially performed to date but analysing the distribution of isolates associated to symptomatic plants already reveals a link between the geographical origin of the isolates and their taxon of belonging. This first result argues for the identification of locally adapted biocontrol agents. Of the taxa already identified, *Fusarium*, *Rhizoctonia*, *Alternaria*, *Epicoccum*, *Trichoderma*, the genus *Fusarium* is the most represented and includes the species *F. solani* and *F. oxysporum*. The specificity of the interaction between the selected isolates and the orobanches, and more particularly *Phelipanche ramosa*, should be finely characterized to avoid that the potential biological control agents affect other plants than the targets.

### **Getting to the roots of black-grass control: Crop-weed allelopathic interactions in *Alopecurus myosuroides*.**

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Black-grass (*Alopecurus myosuroides* L.) is a serious weed of cereals in much of northern and western Europe. The reliance on herbicides as a control method has led to the evolution of multiple herbicide resistance in black-grass populations, currently spanning up to seven modes of action. However, before alternative control methods can be developed and deployed, a clearer understanding of black-grass biology, ecology and biotic interactions is needed. This preliminary study was conducted to determine the effect of both black-grass root exudates and known cereal crop and grass root exudate compounds with allelopathic potential on seed germination and seedling growth of black-grass. Root and shoot growth and seed germination of black-grass were measured in Petri-dish bioassays where freeze-dried, hydroponically-collected black-grass root exudates from herbicide-resistant and herbicide-susceptible populations, and a variety of known allelopathic compounds commonly exuded by the roots of competing crop plants (*meta*-tyrosine, DIMBOA, MBOA, BOA, AMPO, and APO) were applied. Our preliminary results suggest that black-grass root exudates may exhibit some auto-toxicity, potentially resulting in self-regulation at high black-grass densities. *Meta*-tyrosine was also inhibitory to black-grass ( $ED_{50}=295\mu\text{M}$ ). Although less effective, BOA was found to be the most potent benzoxazinoid inhibitor of black-grass root growth and seed germination ( $ED_{50}=561\mu\text{M}$ ). DIMBOA was less effective, but still inhibitory ( $ED_{50}=635\mu\text{M}$ ). MBOA, AMPO and APO appeared to have little to no effect on black-grass development. These results provide the rationale for further examination of these compounds as alternative control methods for black-grass, with allelochemicals being developed as novel natural-product inspired herbicides, or through the enhanced delivery of allelochemicals by cereal and cover crops.

## Optimization and Mutation for High Yielding of Herbicidal Compound HP1 by *Streptomyces achromogenes* KRA2064

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The amount of secondary metabolites produced from wild strains of *Streptomyces* is very low to commercialize. Therefore, it is necessary to develop strategies to achieve hundred times greater yield of metabolites. Improvement of strains and cultivation procedures to obtain high yields have been one of the main objectives in the strain development for industrial research. A wild type *Streptomyces achromogenes* KRA2064 showed a strong herbicidal activity on various weed species and an optimal concentration of herbicidal compound (HP1) produced was more than  $\geq 60 \mu\text{g mL}^{-1}$ . Optimization of culture condition and mutagenesis was conducted to increase the level of herbicidal compound (HP1) production from *S. achromogenes* KRA2064. The optimization for a high yield of HP1 was done by on the basis of culture time, temperature, aeration, agitation, carbon source and nitrogen source. *S. achromogenes* KRA2064 showed maximum yield of HP1 at pH 7, 200 rpm, 27°C, incubation time of more than 42 hours in the medium having potato starch as a carbon source and soybean meal as a nitrogen source on the Jar-fermenter. Additionally, spore of the strain KRA2064 were exposed to UV radiation for physical broad spectrum mutagenesis and to NTG (N-methyl-N-nitro-N-nitrosoguanidine) for chemical mutagenesis. The mutant strain M-289 was obtained with a production titer of HP1 reaching  $256.8 \mu\text{g mL}^{-1}$ , which was increased by 311% in comparison with that of the parent strain. These results can be applied for efficient production of herbicidal compound HP1 as a lead molecule for a more efficient herbicide.

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***Aceria artemisiifoliae* and *Ophraella communa* two natural enemies of *Ambrosia artemisiifolia* occurring in Europe**

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*Ambrosia artemisiifolia*, common ragweed, is an annual herbaceous of the family Asteraceae, native from North America regions, considered invasive in Europe, parts of Asia and Australia. The success as invasive species is probably due to its ecological amplitude and the ability to colonize and dominate disturbed agricultural and anthropic areas. For these reasons, it is an agricultural weed competing with cultivated crops, but the main concern is related to its large production of highly allergenic pollen that induces health respiratory problems among human populations.

In Europe, two natural enemies have been recorded associate with common ragweed: *Aceria artemisiifoliae* (Acari: Eriophyoidea) and *Ophraella communa* (Chrysomelidae: Galerucinae), pointing out the chance that potential biocontrol agents of this invasive species are already present in the Palaearctic Region.

*Aceria artemisiifoliae* is an eriophyid mite, which seems to prevent or at least reduce male flowers development and hence pollen production, but also results in a decrease of the number of viable seeds. Carrying out geographical surveys of an invasive target species is the first step in the selection of perspective agents to introduce into weed biological control programs. First surveys regarding the distribution of this eriophyid mite species are here presented.

*Ophraella communa* is a leaf beetle of American origin accidentally introduced in northern Italy and southern Switzerland. Field data shows an important impact of this leaf beetle on *A. artemisiifolia* plant population density, suggesting a possible gregarious feeding behavior of the insect. Laboratory biological observations, associated with behavioral bioassays are described, providing information on the life cycle and feeding and oviposition behavior of *O. communa*. In addition, electrophysiological recordings by means of gas chromatography coupled with electroantennographic detection (GC-EAD) have been carried out in order to investigate the chemical ecology behind the intraspecific and interspecific interactions of *O. communa*.

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**Does seed predation by carabids contribute to biological weed control? A simulation study.**

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Weed seed predation by carabid beetles (*Coleoptera*, *Carabidae*) may contribute to weed control in crop fields but to date, evidence of weed regulation by carabids is scarce. Predation rates are driven by several factors (crop management techniques, habitat quality, pedoclimate, carabids' abundance and activity and their weed seed preferences). Models are needed to evaluate the impact of weed seed predation on multi-annual weed dynamics, and thus on crop production and weed biodiversity. We developed a predation model which was added to an existing weed dynamics model (FlorSys, Colbach *et al.*, 2014). The model was built from literature data and previous experiments. It simulates the effects on daily predation rates of management techniques, vegetation cover, climate, carabids' intra-annual abundance variation and in-field weed seed preferences of carabids. A sensitivity analysis of predation to model parameters was run to identify the parameters that have the most impact on predation and are thus required to be accurately estimated. Simulations were run to evaluate the impact of weed seed predation on crop production and biodiversity in ten cropping systems. The cropping systems were based on a rapeseed/wheat/barley rotation, differing in rotational crop diversity, herbicides, ploughing and tillage frequency.

Including predation by carabid beetles in the simulations only marginally changed multiannual weed dynamics. Effects of predation on crop production and weed biodiversity were rare and depended on the cropping system. Predation decreased flora species richness in the ten cropping systems because the model assumed that carabids preyed their preferred weed species rather than the most frequent ones. To correct this bias, we plan to include the effect of seed density dependence on predation to the model. We will simulate a wider range of cropping systems to conclude on the contribution of seed predation by carabids in the biological regulation of weeds.

## **Influence of plant decomposition products of weeds on watermelon seed germination and early growth**

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The main objective of this study was to investigate the influence of decomposition products of some weeds (*Abutilon theophrasti* - ABUTH, *Ambrosia artemisiifolia* - AMBAR, *Datura stramonium* - DATST and *Xanthium strumarium* - XANST) on seed germination and early growth of watermelon.

The experiment was set up under controlled conditions. Fresh plant material (leaves, stems, roots) were cleaned several times by tap water, chopped into 1cm long fragments, and mixed with 1 kg of soil (40 g of plant material per 1 kg of soil). The prepared substrates were poured into plastic pots of 14 cm diameter and moistened with 250 ml of water. The substrates were covered with filter paper to prevent evaporation, and kept so for 20 days. After that period, 10 watermelon seeds were sown in each pot. The pots were placed in a climate room under the following conditions: 14h/10h photoperiod, 26/21°C (day/night) temperature and 300µE/m<sup>2</sup>s light intensity. Plants grew for 28 days, upon which period the following parameters were measured: the number of emerged seedlings, fresh and dry weight and height of plants and root fresh weight and root length.

The percentage of emerged seedlings ranged from 92.5-100% for ABUTH, DATST and XANST, while it was lower (65-85%) for AMBAR. The measured root parameters were less sensitive than the fresh and dry weight or height of plants. Root decomposition products of weeds were found to have the highest inhibitory effect (ABUTH:16-35%; AMBAR: 3-46%; DATST: 1-54%; XANST: 47-81%) on measured parameters. Also, stem products caused reduction of plants growth (ABUTH:38-46%; AMBAR: 10-29%; DATST: 13-30%; XANST: 12-25%), while the decomposition products of the leaves showed mainly a stimulating effects (ABUTH: 6-56%; AMBAR: 9-46%; DATST: 3-56%; XANST: 11-44%).

## **Stylosanthes reduces weed infestation in upland rice cropping systems in the Mid-West of Madagascar**

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In the Mid-West of Madagascar, upland rice is highly suffering from low soil fertility and weed pressure. Farmers cannot afford herbicides and rely entirely on a labour-intensive hand weeding. Recently, a no-till system with stylosanthes, a legume cover crop managed as living mulch, has been introduced. It has been proven to enhance soil fertility, but its effect on the weed community was not yet studied. This work aims at assessing the effect of stylosanthes on weed abundance, weed composition, and rice yield. Field experiments were conducted at Ivory station, Mid-West of Madagascar (19°33.29'S, 46° 24.913'E) in 2016 and 2017. Two factors were tested in a split-plot design with 6 replications: main plot factor was soil management (conventional tillage [CT] vs. no-till with stylosanthes [NT]), and sub plot factor was fertilization (manure [F1] vs. manure + NPK + urea [F2]). Weedy and weed-free plots were set up in each factor combination. Stylo-plots (rice and stylosanthes alone, weeds were removed) were set up only in NT, to test potential competition between stylosanthes and rice. Data collected were total weed cover at 60 DAS, rice yield and weed biomass at harvest (grass weeds, broadleaved and sedge separately). Fertilization did not significantly affect total weed cover. Total weed cover was around 65% lower in NT than in CT in both years. Weed biomass at harvest showed that this reduction was mainly due to a decline of grass weeds, as they were the most important functional group in all systems. In CT, rice yield losses due to weeds were around 50% and 85% respectively in 2016 and 2017 showing an important year effect. In NT, rice yield losses due to stylosanthes + weeds were around 50% in both years. Rice yield loss due to stylosanthes alone was not significant.

## **Allelopathic activity and isolation of allelopathic active substances in *Lamium amplexicaule* L. extracts**

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*Lamium amplexicaule* L. is an annual weed belonging to the Lamiaceae family. It is categorized as an invasive alien weed spreading through fields, pastures, and gardens. The invasive characteristic of this weed indicates it is allelopathic. However, the allelopathic activity of *L. amplexicaule* is not well studied. Therefore, the objectives of this study were to evaluate allelopathic activity of *L. amplexicaule* and to isolate allelopathic active substances in the plant extracts. The aqueous methanol extracts of *L. amplexicaule* inhibited the shoot and root growth of cress, lettuce, burdock, Italian ryegrass, barnyard grass, and foxtail fescue, where the inhibition was extract concentration dependent. The concentrations of the extracts required for 50% growth inhibition (*I*<sub>50</sub> values) were in the range of 46.9–937 and 38.5–323 mg fresh weight equivalent extract mL<sup>-1</sup> for the shoots and roots of all the test plants, respectively. On the basis of *I*<sub>50</sub> values, cress shoots and lettuce roots were the most sensitive, while barnyard grass was the least sensitive to the extracts. The extracts were then separated by columns of silica gel, Sephadex LH-20, and C<sub>18</sub> cartridge. In those separation steps, allelopathic activities of all fractions were determined by bioassay and the active fractions were further purified. Two allelopathic active substances were finally isolated by HPLC. The present results suggest that *L. amplexicaule* may have allelopathic activity and contain at least two allelopathic active substances. These substances may play an important role in allelopathic activity of *L. amplexicaule*.

## **Allelopathic effect of aqueous extracts of weeds on tomato seed germination and seedling growth**

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The main objective of this study was to investigate the allelopathic effect of some weeds (*Abutilon theophrasti*-ABUTH, *Ambrosia artemisiifolia*-AMBAR, *Datura stramonium*-DATST and *Xanthium strumarium*-XANST) on seed germination and seedling growth of tomato.

The experiment was set up under controlled conditions. Water extracts were made from fresh plant material (roots, stems and leaves). Fresh plant samples were cleaned several times by tap water, roots were chopped into 1 cm long fragments, while stems and leaves were grinded, soaked and kept in deionized water for 24 h at 4:10 ratio (250 g fresh material in 1000 ml water). The extracts were filtered, and each initial extract represented 100% concentration and was further diluted to obtain 75%, 50%, 25% and 10% concentrations. Deionized water was used as the control. Twenty-five disinfected tomato seeds were placed into each petri dish and 10 ml of aqueous extract was added to each dish and kept in darkness in an incubator (Binder CE) at 25°C. After the period of 10 days, the percentage of germination was calculated and seedlings length was measured. All trial variants were performed in four replications and the trial was repeated twice.

The obtained data showed that the major source of allelochemicals is leaf, than stem and root. The root water extracts of all tested weeds had no inhibitory effect on the seeds germination and seedlings growth of tomato. The stem stock solution and its two dilutions (50 and 75%) caused the inhibition of seeds germination (ABUTH: 51-100%; AMBAR: 66-100%; DATST: 16-31%; XANST: 10-64%) and seedlings length (ABUTH: 78.5-100%; AMBAR: 62.1-100%; DATST: 8.2-69.3%; XANST: 15.7-69.6%). The strongest inhibitory effect was detected for leaf water extracts (stock and all dilutions) for all tested weeds. The reduction of seedlings length ranged from 6.5 to 100%, while the inhibition of seeds germination was 11-100%.

## Potential of *Streptomyces achromogenes* KRA2064 as a New Biological Agent for Weed Control

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Metabolites produced by *Streptomyces* sp. are being studied as possible herbicides or herbicidal adjuvants to develop biological agents that are easily degradable and environment friendly. Therefore, many isolations and screening of *Streptomyces* metabolites have been conducted to find microbial metabolites with bioherbicidal potentials. In the course of our screening of bioherbicidal agent, the isolate KRA2064 had a significant herbicidal activity against a grass weed *Digitaria sanguinalis*. According to the result from 16S rDNA sequence comparison with the close strains, the isolate was identified as a *Streptomyces achromogenes* KRA2064. The foliar application of the culture broth of KRA2064 showed a strong herbicidal activity for grass weed species, *D. sanguinalis*, *Panicum dichotomiflorum*, *Sorghum bicolor*, *Echinochlia crus-galli*, *Agropyron smithii* and broad leaf weed species, *Solanum nigrum*, *Aeschynomene indica*, *Xanthium strumarium* and *Calystegia japonica* in a greenhouse condition. Foliar application of the culture broth of KRA2064 showed phytotoxic symptoms of wilting or burn-down of leaves and stunting and finally plant death. Herbicidal compound was purified by HP20, C<sub>18</sub>, Sephadex LH20 column chromatography and high performance liquid chromatography (HPLC). The structure of active metabolites HC1 was identified by electrospray ionization mass spectra (ESI-MS), <sup>1</sup>H-, <sup>13</sup>C-NMR and 2D-NMR spectral data analyses. These results suggest that *S. achromogenes* KRA2064 producing bioherbicidal metabolites can be developed as a biocontrol agent for weed control or may provide as a lead molecule for a more efficient herbicide.

## Herbicidal effects of emulsions with peppermint or caraway oils against maize and *Echinochloa crus-galli*

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Essential oils are a promising source of allelopathic compounds. There is not enough information regarding the allelopathic potential of essential oils foliar-applied on the acceptor species and therefore, this study was performed. In this experiment, we assessed the phytotoxic effects of water emulsions containing peppermint or caraway oils at two concentrations and with the addition of the commercial adjuvant AtpolanBIO EC80, composed mainly of fatty acid methyl esters of rapeseed oil. A pot experiment was set up in the spring of 2017. Seeds of maize and *Echinochloa crus-galli* were sown into seedbeds. Emulsions with essential oils or with the adjuvant only (control) were foliar sprayed on tested plants at BBCH 13–14. Two days after spraying (DAS) with emulsions containing high doses of essential oils, the aboveground parts of three plants of each species were lyophilized and subjected to metabolomic analyses using gas chromatography/mass spectrometry. The compounds were identified by comparing unknown mass spectra with reference spectra from several commercial libraries. Relative quantification of metabolites was based on a pre-added internal standard (ribitol). The remaining plants were cut at 7 DAS, and the plants injury percentage, plant length, and dry mass were measured. Our results showed that *E. crus-galli* was more susceptible to treatment than maize. Emulsions with peppermint oil were more effective against *E. crus-galli* than emulsions with caraway oil. Principal component analysis and heatmap visualization of metabolomic data showed distinct segregation between control and treated plants for both maize and *E. crus-galli*. Treatments carried out on *E. crus-galli* plants significantly affected a higher number of pathways (impact  $\geq 0.5$ ). In particular, both caraway and peppermint essential oils affected alanine, aspartate, glutamate, and galactose metabolism. In conclusion, the water emulsions of peppermint oil and AtpolanBIO could be used via foliar spraying as effective agents for controlling *E. crus-galli*.



**Plant pathogenic fungi: an excellent and unexplored source of potential natural herbicides**

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*Colletotrichum* is a fungal genus including hundreds of species particularly pathogenic and harmful to cereals, legumes, fruit trees, and vegetables. Some species infect ornamental plants and weeds, too. The genus was recently considered the eighth most important group of plant pathogenic fungi in the world, on the basis of scientific and economic importance. These plant pathogens are primarily responsible for anthracnose diseases, the symptoms of which include necrotic spots on leaves, stems, flowers, and fruits. Secondary metabolites are involved in symptoms and disease development, and some of those metabolites can have novel structures or mechanisms of action, thus representing potential natural herbicides. The phytopathological importance of the genus has led to extensive studies on its pathogenesis, morphology, genetics, physiology, host range, and disease life cycle, not associated to a deep research on the production of secondary metabolites. Thus, a very wide survey was carried out on several tens of strains belonging to this genus, evaluating their capability to produce metabolites with potential herbicidal activities. From one strain of *Colletotrichum higginsianum* several novel compounds were identified, namely colletochlorins E and F, and a new tetrasubstitute dindolylidenepyranone named colletopyranone, together with other known metabolites, such as 4-chloroorcinol and colletopyrone. These metabolites, in particular colletochlorin F and 4-chloroorcinol, caused clear necrosis on plant leaves when applied by puncture, and proved to be active on photosynthesis of *Lemna minor* and on *Phelipanche ramosa* seed germination. From one strain of *Colletotrichum gloeosporioides*, isolated from infected leaves of *Ambrosia artemisiifolia* three phytotoxic compounds causing leaf necrosis, namely colletochlorin A, orcinol and tyrosol, were identified. The aim of the present communication is to illustrate the more recent findings in this research field, showing chemical and biological properties of the discovered metabolites, and to discuss the possible developments of those and other fungal metabolites as natural herbicides.

## Effectiveness of a natural fertilizer in controlling the parasitic weed *Phelipanche ramosa*

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The parasitic weed *Phelipanche ramosa* (commonly named branched broomrape) is responsible for enormous yield losses of several crops in the Mediterranean basin and in many other European countries, attaching in particular the Solanaceae, with other crops such as rapeseed, cabbage and hemp now increasingly affected. Traditional weed management methods can provide only a modest or even a null control. Difficulties in controlling parasitic weeds are due both to the large amount of seeds produced that can remain viable, even in the absence of a host, for many years, and for the physiological and biological properties of the parasite. The seed germinates only if stimulated by host root exudates and produces a germ tube that, after attaching the host root, develops a haustorium that penetrates the root and forms a tubercle. This is followed by the withdrawal of nutrients, water, and photosynthates from the host. Furthermore, broomrapes have a long underground phase, so that when they emerge most of the damage has already been produced. Recently, by chance a vegetable organic fertilizer (commercial name: ORODEM2) with radical action commercialized for tomato fertilizing proved to have a good effectiveness in reducing crop parasitization and in increasing crop yield. Thus, a number of experiments were performed in naturally infested fields by using different doses and time of application. Efficacy of treatment was evaluated in terms of percentage of infested host plants and number of emerged parasite shoots. Moreover, in order to identify the natural metabolite(s) responsible of the observed efficacy, the purification of the vegetable fertilizer was performed. From the organic extract some metabolites having inhibitory capability on *P. ramosa* seed germination and tubercle development were purified by HPLC. Their identification by LC-MS, and their biological characterization is in progress. The preliminary results of field effectiveness and chemical and biological characterization of the vegetable fertilizer will be presented.

### **Effects of Arbuscular Mycorrhizal Fungi Inoculation on Redroot Pigweed on Sesame under salt stress**

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The aim of this research was investigated of arbuscular mycorrhizal fungi (*Glomus mossea*) on redroot pigweed (*Amaranthus retroflexus* L.) and Sesame (*Sesamum indicum*) Darab cultivar competition under salt stress. The greenhouse experiment was laid out as a factorial based on randomized complete design with three replications at Faculty of Agriculture, Shiraz University, Iran in 2015. The treatments were consisted of salt stress (S0=water as control, S1=0.8, S2= 7 and S3=14 ds/m Of NaCl+CaCl<sub>2</sub>), fungi (F0: control without inoculation, F1: inoculation with *Glomus mossea*) and redroot pigweed (W0: weed free, w1: weedy). The results showed that interactions effect of different levels of salt stress, mycorrhizal fungi and weed on Sesame physiological traits was significant ( $P < 0.01$ ). Mean comparison showed that increasing of salt stress decreased grain yield, plant height and leaf area index significantly. Maximum value of all traits achieved by S1F1W0 treatment. Application of 14 ds/m salt stress stopped redroot pigweed growth, while this treatment increased inoculation of Sesame root with mycorrhizal fungi under severe salinity. Thereby, it showed a better performance on all traits. In general, it seems that application of mycorrhizal fungi can improve morphological and agronomic characteristics of Sesame by increasing nutrient uptake comparison with redroot pigweed. Mycorrhizal fungi inoculation showed significantly increased on all traits compared to non-inoculation plants under different salinity levels. Therefore, the highest biomass and plant height in Sesame were obtained from the inoculation with *Glomus mossea*. Results also indicated that weed biomass and height decreased by increasing salinity stress in non-inoculated treatments. Based on our results, mycorrhizal fungi can decrease the negative effects on weed competition in Sesame through increasing of plant growth under salinity stress conditions.

***Aculops mosoniens* (Acari: Eriophyoidea) a potential biocontrol agent of *Ailanthus altissima***

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*Ailanthus altissima*, commonly called tree of heaven, is a fast-growing deciduous tree, native to China. It is a serious invasive species worldwide (Europe, Asia, South Africa, South Eastern Australia, North America) of disturbed urban sites, competing with autochthonous flora and causing structural damages to railways, roads, buildings. In addition in rural areas, it invades cropland, fencerows, forest edges and rocky areas.

Total eradication of this alien species is problematic and efforts have to be addressed for the ecological preservation of the ecosystems, by the use biological and integrated control approaches. Among the potential candidate agents, eriophyid mites play a relevant role, because they are obligate plant feeders with high host specificity.

Few eriophyid species were found to be associated with *A. altissima*, but recently a new eriophyid species has been described in Hungary, and it appears to offer a chance to have a potential biocontrol agent which does not require to be imported. *Aculops mosoniensis* (Ripka & Érsek) forms dense populations on the undersurface of the leaflets, where it is making the lamina narrow, deformed, twisted, with edges folded or rolled downward toward the main vein of the leaflets. Drying and necrosis of the apical parts of the stems have been observed on heavily infested plants; whereas high infestations in young plants are also associated to the premature loss of leaves.

First surveys of the distribution of *A. mosoniensis* are here presented, together with the first data regarding the potential impact of this eriophyid mite species on *A. altissima*.

Based on the induced damages on the host plant, *A. mosoniensis* looks a perspective agent for the biological control of tree of heaven: for this reason field observations and laboratory bioassays on the biology, host specificity and impact of this eriophyid mite on the target plant species are currently under evaluation.

**Phytotoxic and antimicrobial activity of selected Medicinal and Aromatic plants (MAP's)**









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Increasingly recorded cases of resistance of weeds species and phytopathogenic microorganisms to registered herbicides and fungicides respectively, create the need of new solutions to protect crop production. Aromatic and medicinal plants with their bioactive compounds, can provide a potential alternative solution for weed and diseases control. The aim of this study is to investigate the bioactivity of hydrosols of two MAP's, *Origanum hirtum* and *Coridothymus capitatus* originating from the island of Ikaria. Their inhibitory activity was evaluated, in vitro assays, against the weed *Lolium multiflorum* and, the bio indicators *Avena sativa* and *Lemna minor* as well as the phytopathogenic microorganisms *Verticillium dahliae* and *Clavibacter michiganensis* subsp. *michiganensis*. According to methodology, the hydrosols received from plant tissues (leaves and inflorescences) subjected to hydrodistillation, were diluted in various concentrations: 50%, 25%, 12,5%, 6,25%, 3,125% v/v. In petri dishes incubating assays (with 3 replications), the seeds germination, the shoot and the radicle growth of *Avena* and *Lolium* and the fronds growth of *Lemna* in aqueous culture, were measured daily for 14 days. In the case of hydrosols activity on the phytopathogenic microorganisms, measurements of the mycelium growth and the number of bacterium colonies were taken after 7 and 3 days respectively. According to ANOVA analysis, both hydrosols proved to be extremely bioactive, with the *origanum* hydrosol to be more effective compared to *coridothymus* one, against all organisms tested, even in low concentrations.

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