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Optimizing herbicide use in an Integrated Weed Management (IWM) context

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Optimizing herbicide use in an Integrated Weed Management (IWM) context

Local Organizing Committee

Dr. Demos Chachalis, *Benaki Phytopathological Institute*
Dr. Thomas Gitsopoulos, *Hellenic Agricultural Organization-Demeter*
Mr.GiorgosGeorgoulakis, GEOTEE

Scientific Organizing Committee

Prof. Per Kudsk, *Aarhus University* (per.kudsk@agrsci.dk)
Dr. Demos Chachalis, *Benaki Phytopathological Institute* (d.chachalis@bpi.gr)
Dr. IliasTravlos, *Agricultural University of Athens* (travlos@aua.gr)
Prof. Rafael de Prado, *University of Cordoba* (qe1pramr@uco.es)
Prof. Josef Soukup, *Czech University of Life Sciences* (soukup@af.czu.cz)

Session organizers

- **Integrated weed management**
  P. Kudsk and I. Travlos

- **Herbicide resistance**
  R. De Prado, D. Chachalis and M. Simic

- **Invasive weeds / Chemical and non chemical methods**
  M. Riemens, P. Lolas and A. Karkanis

- **IWM and Herbicide use**
  T. Gitsopoulos and C. Damalas

Integrated weed management: The future paradigm

Kudsk P.

Dept. of Agroecology, Aarhus University, Slagelse, Denmark.
(per.kudsk@agro.au.dk)

The on-going loss of active ingredients, the total absence of new herbicide modes of action, the steady increase in the number of herbicide resistant weed biotypes and the emergence of new weed species are serious challenges to the sustainability of the largely herbicide-based weed management strategies adopted by the vast majority of European farmers. Many farmers have reached a crossroads where weed management strategies need to be redesigned.

In June 2014 Directive 2009/128/EU enforcing EU Member States to ensure a sustainable use of pesticides came into force. An important feature of the directive is the requirement that from 1 January 2014 all professional users of pesticides were supposed to comply with the general principles of Integrated Pest Management (IPM). Considering the challenges farmers are facing more and more farmers realise that a weed management strategy relying solely on herbicides is doomed to fail. In this perspective Directive 2009/128/EC with its demand to adopt IPM is very timely.

Adopting integrated weed management (IWM) often involves major changes in the cropping system as the composition of the weed flora is closely associated with the cropping system. No single alternative control method can replace the use of herbicides and IWM typically involves the application of several control methods in a systematic way. IWM is therefore much more knowledge intensive than the current practice.

In recent years more information has become available on the performance of alternative control methods but information on how to combine these methods into effective IWM practices is still scarce. Furthermore many years of access to effective and often cheap herbicides - an easy to apply and very reliable technology - means that a change in the farmers' attitude to weed management is needed for successful implementation of IWM.

In this presentation the principles of IWM will be presented as well as results from previous and more recent research.

IWM: an Industry View

Cornes, D. W.

Syngenta, Bracknell, UK (derek.cornes@syngenta.com)

The grower is looking for as close to 100% weed control as he can achieve but he is also looking to do this in a simple way. This is why selective herbicides have been so successful and why crops modified to tolerate non-selective herbicides dominate in the countries where they are available.

Why would a grower who can get excellent weed control by a simple herbicide spray do anything differently? This is a fundamental question when considering IWM; since it adds complexity, potentially adds cost and may not be as reliable as a chemical spray. In reality, unless practicing IWM brings a clear benefit to the grower, he/she will not be inclined to adopt or sustain IWM.

What constitutes IWM? - Anything that can affect weed control! Cultural techniques, chemicals, correct application timings, scouting / mapping, farm management programmes / data analysis, hand weeding, tillage, biologicals, allelopathy, robots….preferably in some form of holistic programme.

So what are the factors that might make a grower considering introducing IWM? Factors such as cost savings, improved yields, legislation, subsidies and difficulties in controlling weeds (resistance, lack of registered products, product use is restricted), will all encourage growers to look beyond chemical herbicides.

There are, in fact, many growers already managing their fields with IWM, as some of the factors mentioned above are coming into play. Issues such as the management of resistant weeds and weed control in crops where limited numbers of chemical herbicides are registered, force the grower to implement IWM.

This situation will become a reality for more growers over the coming years due to the following factors: resistance to existing herbicide chemistry developing and spreading, no new herbicide mode of action commercialised since the 1980's and no major new commercial herbicide mode of action on the 10 year horizon. Older herbicides are being withdrawn due to regulatory issues and new herbicides are becoming more difficult to register, especially in Europe. As the development of a new active ingredient costs around $250m and has 10 year development cycle, only uses which justify this investment, and the considerable risk involved, will be considered. The result is that some weeds are now almost impossible to control with chemicals alone and there are some crops with no good herbicides registered. This situation is only likely to deteriorate further.

The agrochemical industry is rapidly adapting to this scenario and is now developing IWM strategies as part of the product offer. The benefits to industry include: maintaining valuable products by limiting resistance development, maintaining products by allowing lower use rates where there are regulatory issues and the creation of differentiated offers by moving from selling purely chemicals to providing integrated weed control solutions for the grower.

1st SESSION: INTEGRATED WEED MANAGEMENT

Optimization of herbicide use: Results from pilot field projects in major crops in Greece

Demosthenis Chachalis

Benaki Phytopathological Institute, Laboratory of Weed Science,
S Delta 8, 14561, Athens.
d.chachalis@bpi.gr

In this presentation, results from 3 major projects (such as a LIFE+ EcoPest (www.ecopest.gr, a LIFE+ HydroSense (www.hydrosense.org and a national GSRT project, BreedSeed) will be presented. The objectives of those projects were to demonstrate at field scale effective means of optimization of herbicide use aiming to ameliorate the environmental effects of herbicides at field and catchment level. The EcoPest project (1.000 ha, at the Kopaida plain) field pilot studies were made in optimization of herbicide use for 3 years in cotton (mainly), corn and plum tomato. The HydroSense project was applied for 3 years in pilot sites in Thessaly region in cotton aiming to incorporate precision agriculture into optimization of herbicide use. The BreedSeed project applied for 2 years in several sites in cotton and corn aiming to determine the effects of seed vigor and first growth on the competition with the weeds. In the field pilot sites, broadcast herbicide applications were substituted with band applications for both PPI and PRE, after suitable conversions in the spraying equipment. In addition, a new system utilizing sensors and spraying was used in POST-directed glyphosate application in cotton. Significant reductions on herbicide (both PPI and PRE) amounts were recorded (approximately 60%), without any weed failures in the crop. Reductions of POST-directed glyphosate were 10-90%, depending on purple nutsedge density, with improved control on the weed species. Components of seed vigour and early growth were determined that affected the competitive ability of the crop against the weeds. Results from those projects, highlight that optimization of herbicide use proved to have a positive effect ameliorating environmental impact of herbicides in vulnerable agro ecosystems.

Contribution of crop rotation to herbicide weed control in maize

Simic M., Spasojevic I., Dragicevic V., Brankov M.

Maize Research Instiute Zemun Polje, Belgrade, Serbia (smilena@mrizp.rs)

Crop rotation is effective and basic measure within IWMS. By rotation of row and narrow crops and systems of measures for their production, including application of herbicides with different mode of action, weed community has no opportunity to become adapted and stabile. In Serbia, there are three major maize growing systems: continuous maize cropping (15%), two crop rotation (maize -winter wheat - 60% and maize - soybean - 15%) and three crop rotation (maize - winter wheat - soybean - 5%). Furthermore, in smaller fields, maize is grown after some other crops such as alfalfa and other legumes, vegetables, pastures etc. (5%). Extended rotations involving legume crops reduce N inputs, increase maize grain yield and are more sustainable than current short-terms rotations. On the other hand, herbicides in recent time have to fulfil many tests during registration process in order to become approved as more environmentally friendly. They need to be effective and selective and do not make serious changes in weed community balance and encourage some troublesome weed species such as alien or perennial weeds, or emphasize resistant biotypes. The aim of the research was to test effectiveness of combined application of crop rotation and herbicides that are applied in recommended and reduced rates on maize weed control. A field experiment was conducted during six years, 2009-2014, as a split-plot trial on slightly calcareous chernozem on the experimental field of the Maize Research Institute Zemun Polje, Belgrade, Serbia. The basic treatment was a plant production system: maize continuous cropping (MC), maize-wheat rotation (MW) and maize-soybean-wheat rotation (MSW). The hybrid ZP606, wheat variety Takovčanka and soybean variety Lana were conventionally sown within optimal periods in all production systems. Conventional tillage was used in the trial. A total of 30 t ha^{-1} of manure was incorporated in autumn of 2008 and then every third year in maize monoculture and MSW. In autumn of 2008 and 2010, 20 t ha^{-1} of manure was incorporated in two-crop rotation variant and every second year after. Different herbicide rates represented treatments in sub-plots. After sowing and prior to maize emergence the combination of isoxaflutole and acetochlor (Merlin 750-WG+Trophy 768-EC) was applied in two rates: HR- recommended (105 g a.i. + 1536 g a.i.) and ½ HR - half of the recommended rate (52.5 g a.i. + 768 g a.i.), while herbicides were not applied in the control. Each treatment had four replications. In wheat and soybean usual combination of herbicides for broadleaf and grass weed control was applied in whole variant. Weed estimation was done four (I estimation) and six to seven weeks (II estimation) after the application of herbicides. Samples were drawn randomly by the one square meter. Number of weed species and weed individuals per species and fresh biomass of manually uprooting weeds were estimated in maize.

Weed association was composed of eleven most distributed species in all cropping systems. Between them, annual broadleaf species *Datura stramonium*, *Solanum nigrum* and two species from genera *Chenopodium* and *Amaranthus* were present. Perennials *Convolvulus arvensis* and *Sorghum halepense* were dominated, almost uncontrolled species. Number of individuals of *C. arvensis* were even higher after some years, especially in maize continuous cropping. Weed individuals and biomass abundance were significantly lowered with herbicide application in all years and cropping systems. In most of the years, half of the full dose appears to perform nearly as well as the full dose what is especially obvious during first evaluation i.e. four weeks after herbicide application.

New Minor Crops in Greece-New IWM challenges

P.C. Lolas

School of Agricultural Sciences, Dep. of Agriculture, Crop Production and Rural Environment. Univ. of Thessaly, Fytokou Str. GR-384 46, Volos
lolaspet@gmail.com

Greek agriculture nowadays undergoes a diversification stage, considered as very crucial for reforming the Greek economy. Most of the farmers in Greece still continue to base their income on traditional crops. However, the last 5-8 years there is an increasing every year number of growers who have turned to new crops. Pomegranate (*Punica granatum*) sea-buckthorn (*Hippopfae rhamnoides*), blueberries (*Vaccinium* spp.), goji berry (*Lycium barbarum*), stevia (*Stevia rebaudiana*), aronia (*Aronia melanocarpa*), and a number of aromatic-pharmaceutical species are now grown in many places in Greece as minor crops. Weeds are a serious problem also for minor crops as with all traditional crops. Especially in minor crops, weed control is a challenge since at present there are no herbicide(s) registered and weed control is only based on cultural practices. Effective integrated weed management (IWM) cannot depend only on cultural or other non chemical methods. In many cases the presence and seriousness of certain weeds, e.g. perennials, requires the use of effective herbicides. This, in combination with yet no registered herbicides for the above minor crops implies the urgent need first to find through field experiments effective and selective herbicides and secondly the registration of some of them for these minor crops. Obviously, in these field trials one of the main objectives should be the optimization of herbicide use regarding the less adverse effects on the environment (including man health), the rates and the application time. In Greece such data are available only for stevia. It is suggested that research institutions should be financed by Ministry of Agriculture. E.U. and/or private organizations to carry out the relevant IWM trials on all above new minor crops in Greece.

Keywords: Pomegranate, sea-buckthorn, blueberries, goji berry stevia, aronia

Control of black grass (*Alopecurus myosuroides* Huds.) by integrated crop management

Nilsson A. TS.[1] & Hallqvist H[2]

[1]*Swedish University of Agricultural Sciences, Alnarp, Sweden.*
[2]*Swedish Board of Agriculture, Alnarp, Sweden*
(anders.ts.nilsson@slu.se)

Black grass is a weed problem that is increasing in prevalence in Sweden. Increased cultivation of winter crops as well as cropping systems with reduced tillage favours the occurrence. Black grass easily develops resistance to herbicides and resistant black grass populations are present in many European countries, so also in Sweden. Control strategies that counter act herbicide resistance include greater use of cultivation measures such as plowing, crop rotation, delayed sowing, cultivar with increased weed competition but also decreased dependence on high-risk herbicides. However, it is not likely to change in herbicide use alone can reduce the risk of herbicide resistance enough. The aim of this study is to evaluate and give indications on how important the choice of cultivar, seed rate and sowing date are to increase the crop competitive ability and possibility to reduce the problems of black grass in winter cereals and as a consequence of that also the reliance on herbicides This study examines crop management measures competitiveness against black grass and other weeds in winter wheat by combinations of **cultivars** (2) with different competitiveness, stand density/**seed rates** (2) and **sowing dates** (2), totally 8 combinations. The different combinations need for additional chemical control was evaluated by that the trials was designed as split – plot experiments where **herbicide treatments** (3 + untreated control) was performed across trials plots. The trials are designed as block-trials with four replications and the project is intended to be carried out for three years with three trials each year. The results from the first project year, season 2013-14, has shown that crop management measures can have a significant impact on the population of black grass .Of the three tested cultivation factors seems the sowing date to have the highest effect. Two weeks later sowing (14 Sep -> 1 Oct) resulted in -65 % reduction of plant number of black grass, -77 % of plant weight and also -77% of ear number, in plots without herbicide use. A low dormancy and early germination of the black grass seeds in the summer/autumn 2013 can likely explain the high effect of sowing date. The effect of choice of cultivar and seed rate showed lesser, but useful, impact on the black grass population. Comparing combinations with, theoretically, the “lowest” (early sowing, cultivar with low competitive ability and low seed rate) and the “highest” (later sowing, cultivar with high competitive ability and high seed rate) effect indicates the” potential” of the crop management measures. Based on this comparison the “potential” of cultivation factors (without herbicide assistance) reduces the plant number of black grass with -81%, weight with -87% and ear number -83%. At the same time the number of wheat plants increased with +48%, wheat ears with +79% and yield with +30%. Crop

management measures can be useful tools in sustainable control of black grass.

Integrated weed control by inter-row hoeing and intra-row herbicide treatment in annual crops

Lundkvist A[1], Nilsson A TS[2], Verwijst T[1], Algerbo P-A[3], Gilbertsson M[3], Ståhl P[4], Hansson D[2], Andersson A[2]& Stenberg M[5]

[1] *Swedish University of Agricultural Sciences, Uppsala, Sweden.*
[2] *Swedish University of Agricultural Sciences, Alnarp, Sweden.*
[3] *JTI - Swedish Institute of Agricultural and Environmental Engineering, Lund, Sweden.*
[4]*The Rural Economy and Agricultural Societies, Vretakloster, Sweden.*
[5]*Swedish University of Agricultural Sciences, Skara, Sweden.*
(anders.ts.nilsson@slu.se)

Herbicide application is the most commonly used weed control method in annual crops. However, the number of available herbicides is decreasing. At the same time, the development of herbicide resistance is increasing in Europe. Also, farmers within the European Union have to apply integrated pest management (IPM) from 2014. This has increased the need for development of alternative weed control measures with reduced amounts of herbicides. The aim of this project was to develop and evaluate an integrated weed control strategy against annual weeds. Inter-row hoeing, intra-row spraying, and combination of the two methods were compared with conventional broadcast spraying. New equipment for intra-row spraying was developed. A sprayer boom was constructed and sprayer nozzles were mounted on the boom. The sprayer boom together with the sprayer tank and pump were installed on an inter-row hoe and seeder. The spraying width was 8-10 cm, and the sprayer nozzles were placed about 30 cm above the crop rows. Field experiments were performed 2012-2014 in the southern parts of Sweden in spring oilseed rape, winter oilseed rape, winter wheat, and faba beans. Preliminary results showed that the combination of inter-row hoeing and intra-row spraying gave similar weed control effects and crop yields in the studied crops as broadcast spraying. We conclude that the inter-row hoeing and intra-row herbicide treatment diminish the overall use of herbicides in comparison with broadcast spraying, while having similar weed control effects.

Effect of site-specific weed management on crop yield and weed populations

Hamouz P.

Czech University of Life Sciences Prague, Czech Republic.
(*hamouzp@af.czu.cz****)***

An aggregated distribution pattern of weed populations provides opportunity to reduce the herbicide application if site-specific weed management (SSWM) is adopted. Accurate threshold values are, however, needed for reliable use of this method. Presented research study was focused on evaluating the effects of SSWM on crop yield and development weed populations over a 4-year period. SSWM was used on a 3.07 ha experimental field in central Bohemia during 2011–2014. Winter wheat and winter rape were grown in this period. The experimental area was split into cells of 6 × 10 m. A total of 512 cells were arranged into 16 blocks, which allowed the randomization of four treatments in four replications. Treatment 1 represented blanket spraying regardless of weed infestation whereas the treatments 2 – 4 consisted of SSWM with increasing thresholds used for individual weed groups. Weed infestation was evaluated in spring of each year prior to post-emergence herbicide application. The density of each weed species was evaluated manually by counting individual weeds in four samples taken in the central part of each cell. Treatment maps were created for each weed group based on weed abundance data and treatment thresholds. Following thresholds were used in treatments 2; 3 and 4 respectively: 0.2; 0.5 and 1 plant m^{-2} for *Galium aparine* L. and *Cirsium arvense* (L.) Scop, 5; 10 and 15 plants m^{-2} for *Tripleurospermum inodorum* (L.) Schultz-Bip. and for annual grasses. Thresholds of 10; 20 and 30 plants m^{-2} were used for other dicotyledonous weeds. Herbicide application against individual weed groups was performed separately using a sprayer equipped with GPS and boom section control. Differences in yield and population density among treatments were analysed by ANOVA with significance level α=0.1. The yields of winter wheat and winter rape in SSWM treatments varied between 94.6 % and 103 % of blanket treatment and differences were statistically insignificant in all years. SSWM led to significantly increased density of *G. aparine* in 2013 and 2014, and its abundance increased with rising threshold value. Significant differences were found between treatments 1 and 4 in 2013 and 2014. Similarly, density of the *T. inodorum* population increased substantially on SSWM plots in the third and fourth experimental years. Treatment 1 was found to be significantly different from treatments 3 and 4 in 2013 and from treatment 4 in 2014. Annual monocotyledonous weeds were represented mostly by *Apera spica-venti* (L.) P.B. Plant densities of this group were also higher SSWM and increased slightly with increasing threshold value, but the herbicide savings were acceptable here even in last experimental year. The effect of SSWM was insignificant for other weeds. Based on these results, very low SSWM thresholds are recommended for *G. aparine* and *T. inodorum*. Higher threshold could be considered for *A. spica-venti* because of absence of a long-term soil seed bank.

The suppression of weeds by cover crops grown as living mulches in perennial fruit crops

Tzortzi M., Roberts H., Atwood J., Tatnell L.V.

ADAS Boxworth, Battlegate Road, Boxworth, Cambridge, CB23 4NN, UK
(Maria.tzortzi@adas.co.uk)

There are a limited number of herbicide options available in perennial fruit crops in the UK and growers are increasingly looking for alternative or integrated methods of weed control. Cover crops can be used in different field situations as ground cover, green manures and living mulches providing many benefits. Living mulches are low growing crops established either prior to or at the same time as the main crop. Some of the benefits that they offer are to enhance the soil structure (e.g. prevent soil erosion by covering the bare soil, increase organic matter), provide a habitat for beneficial insects and provide weed suppression, which could be particularly useful when herbicide options are limited or unavailable e.g. organic production. It is however very important to select the correct living mulch, as competition for water or nutrients could cause the living mulch to be detrimental to the crop. This three year study is investigating the potential advantages of using living mulches in an apple orchard for weed control efficacy, soil moisture requirement, nutrient status and crop quality. A series of experiments in both outdoor containers and in an established apple orchard, are examining the potential of several species for use as living mulches in perennial crops. The species include *Trifolium repens* (white clover), *Medicago lulupina* (black medic), *Festuca rubra* (creeping red fescue), and *Lotus cornilatus* (birdsfoot trefoil). Results to date demonstrate the high weed (perennial and annual species) suppressive ability of creeping red fescue alone and in a mixture with birdsfoot trefoil. Leguminous species showed no effect on available nitrogen, however results have shown that, white clover requires more moisture. No significant differences were observed in apple yield and quality compared with an herbicide treated control in the year after establishment. This work is part of a Horticultural Fellowship Award 'Weed control in ornamentals, fruit and vegetable crops- maintaining capability to devise sustainable weed control strategies'. It has been funded by Agriculture and Horticulture Development Board, The Horticulture Trades Association and The East Malling Trust.

New technologies in rice cultivation

C. Dramalis*, A. Kalaitzidis, D. Katsantonis, and S. Vizantinopoulos

Hellenic Agricultural Organization – DEMETER, Institute for Plant Breeding and Genetic Resources, Ellinikis Georgikis Scholis, P.C. 57001, Thermi Thessaloniki, GREECE
(dramalis@cerealinstitute.gr)

Global average rice yield should be increased to meet the consumption needs of the growing population. Nowadays, precision agriculture is considered the appropriate technology tool that can make significant contributions to both food security and sustainable development. To this end, the Hellenic Agricultural Organization has conducted three European funded projects since 2011. The SMART-PADDY project (2011-2013) developed a wireless sensor network consisted of low-cost nodes with an autonomous power supply, capable of transmitting real-time EC measurement readings to a local and to a remote server. The system can withstand the harsh environment of the rice fields. Furthermore, the cultivation practices, such as fertilization and herbicide spraying, do not interfere with the operation of the system. The RICE-GUARD project (2013-2016) aims to develop a low-cost in-field heterogeneous wireless sensor network (WSN) to predict rice blast. WSNs can overcome one of the main limitations of current forecasting methods based on weather data, either solely or combined with additional information such as spore counting. Rice blast is the most serious disease of cultivated rice and the pathogenic fungus overseasons on infected rice residues or grain and on weed hosts. ERMES project (2014-2017) aims to develop a prototype of downstream service dedicated to rice sector based on assimilation of earth observations and in situ data within crop yield modelling. The objectives of this service are: a) contribution to the regional authorities for making policies related to rice crop and the new CAP, b) development of sustainable strategies and reduction of the inputs (fertilizers, herbicides, energy) in the field, c) early warning for risks due to biotic (pests, weeds, diseases) and abiotic (drought, salinity, high temperatures) stresses and d) provision of information to the agri-business for making reliable commercial and exportation decisions.

2nd SESSION: HERBICIDE RESISTANCE

Integrated Weed Management as a Tool to Suppress Herbicide Resistance in Weeds

Hamouzová K.; Soukup J.

[1] Czech University of Life Sciences Prague, Prague, Czech Republic.
(hamouzova@af.czu.cz)

Integrated weed management (IWM) focuses on long-term prevention of weeds by managing the ecosystem by combining management approaches for greater effectiveness. It requires the capability to predict weed occurrence and its potential impact on the crop. Since, the weeds are predominantly controlled with herbicides, response of weed to respective herbicide play an important role as well. For weed plants, herbicides are rapid stress event, which initially few individuals can survive. Clearly, herbicide resistance evolution has to be considered in IWM. Herbicide resistance is an evolutionary process dependent on the number of factors, such as population growth rate, genetic diversity, reproduction, agronomic practices and herbicide selection use history, including the dose applied. Herbicide application are not homogenous across fields that some individuals receive reduce dose of herbicide while treated. Additionally, herbicide use doses vary markedly between nations, regions and enterprises, reaching up to 50% lower dose in some country compared to another one. Herbicide properties and dose strongly influences the types of resistance genes that can be enriched. Careful consideration of herbicide selection is important aspect of weed management. Resistant weeds surviving herbicide application use a variety of mechanisms, in the broadest sense we usually divide them to target-site resistance (TSR) mechanisms including increased expression of target protein or structural changes to the herbicide binding site; and non-target-site resistance (NTSR) mechanisms include changes in molecule penetration, translocation, accumulation and binding to the target protein. TSR is often determined by dominant or semi-dominant alleles at a single nuclear gene locus, recent findings revealed that recessive control of TSR is also present. In some of the herbicides groups, the nuclear monogenic control has been identified, while in others it is cytoplasmic. Latest studies showed that NTSR is the predominant type of herbicide resistance associated with higher secondary metabolism in plants originating from complex abiotic stress-response pathways. The perpetual question based upon evidence that higher doses select for major gene opened further research recently. The studies have shown that the relative initial frequency of the major and minor gene alleles affect the response of plant to herbicide dose. No effect due to difference in herbicide dose was found, if the frequency of the major gene allele is high enough then ‘major gene’ mono-allelic resistance occurs first. At reduces herbicide doses polygenic resistance build up if the frequency of the minor gene allele is high enough, compared to the frequency of the major

gene allele. Additionally, the modeling studies showed that lower doses can hasten polygenic resistance but delay monogenic resistance assuming that the evolution of resistance depend on many factors. Anyway, from the farmer's point of view, the negative effects of low doses are more evident, when considering weed density rather than allele's frequency. Recommendations regarding the implementation of IWM usually call for the use of multiple strategies to control weed populations in economical and environmental-friendly way. The most common recommendations to the farmer how to slow down herbicide resistance evolution are to apply mixtures of herbicides with different mode of action. This recommendation is short sided, taking into account that NTSR is often responsible for cross-resistance. The recommendation should follow with the statement that also herbicides in which different metabolic pathways are involved in the degradation should be rotated. Having in the mind, that many of active ingredients will be banned in the near future, the farmer should avoid low-dose herbicide usage to help achieve herbicide sustainability. Plants surviving the low doses increase the risk of enrichment of gene traits leading to herbicide resistance. It becomes evident that herbicides should be used at doses that cause very high weed mortality. More likely the herbicide mixtures would delay the evolution of TSR than herbicide rotations. Often, when there is herbicide resistance present in the weed, using of this respective herbicide in the mixture with another partner is inefficient. The use of nonchemical control strategies is in such cases inevitable to minimize the likelihood of resistance genes being passed to the next generation. In the early phase of selection the resistant phenotypes are not evident in the field and very often the problem is neglected regardless the mechanism of resistance. Due to a late recognition of herbicide resistance in the field, the solution is more difficult and requires additional costs and measures.

Indications of evolving multiple-resistance to glyphosate, glufosinate and oxyfluorfen in the genus *Lolium*

Fernández-Moreno, P.T.[1], Alcántara-de la Cruz, R.[1], de Portugal, J.[2], Calha, I.[3], Costa, J.[4], Brants, I.[5], De Prado, R.[1]

[1]*Department of Agricultural Chemistry and Edaphology, University of Córdoba, 14071 Córdoba, Spain*. [2]*Agrarian Superior College of Beja, Beja, Portugal.* [3]*National Institute for Agricultural and Veterinary Research, Public Institute, Quinta do Marquês, 2780-155 Oeiras, Portugal.* [4]*Monsanto Agriculture Spain, Madrid, Spain.* [5] *Monsanto Europe S.A., Brussels, Belgium.*
(ge1pramr@uco.es)

Glyphosate is the most important and widely used herbicide in the world. In the Mediterranean area, the genus *Lolium* has developed resistance to glyphosate after decades of continued use without integrating alternative weed control practices in perennial crops (olive, citrus and vineyard). Oxyfluorfen alone or mixed with glyphosate and glufosinate herbicide were management options that provided good alternatives for farmers to control broadleaf and grassy weeds. However, farmers did not always implement integrated weed management practices (herbicide active ingredient rotation, mechanical control, biological control) for these solutions. In this study, we confirmed the first case of evolving low-level resistance to glyphosate, glufosinate and oxyfluorfen in *L. multiflorum* and *L. rigidum* in the world. Dose-response assays to determine the herbicide required for a 50% plant growth reduction (GR_{50}) were carried out. According to $GR_{50}$for the *L. multiflorum* populations, the resistance factors (FR) ($GR_{50}R/GR_{50}S$) were 3.66, 3.17 and 2.67 times higher respectively for glyphosate, glufosinate and oxyfluorfen when compared to a sensitive (S) population. Similarly, the FR values for the *L. rigidum* populations were 15.41, 2.78 and 3.25 times higher for glyphosate, glufosinate and oxyfluorfen respectively when compared to S one. Both *Lolium* species showed two similar resistance mechanisms to glyphosate; the 1st due to an amino acid change at position 106 of the EPSPS (Pro to Ala); and the 2nd a reduction in the penetration and subsequent translocation of herbicide in the resistant population. Resistance mechanisms to glufosinate and oxyfluorfen are still unknown.

Keywords: *Lolium*, glyphosate, glufosinate, oxyfluorfen, GR_{50}, TRS, NTRS.

Glyphosate resistant populations of *Conyza* spp. in Greece: current situation, fitness and management

I. Tabaxi[1], I.S. Travlos[1], C. Astrakis[1], P.J. Kanatas[1], E. Tani[1] and D. Chachalis[2]

[1] Agricultural University of Athens, Faculty of Crop Science, Athens, Greece
[2] Benaki Phytopathological Institute, Kifisia, Greece

Globally, the extended use of glyphosate resulted to its reduced efficacy against increasingly problematic weeds, such as *Conyza* spp. This study attempts to describe the current status of glyphosate resistance in Greece and to highlight several proactive and reactive approaches for the avoidance of further spread and management of this serious problem. Moreover, ecological fitness of some glyphosate -resistant and -susceptible populations (GR and GS, respectively) is also evaluated. Data from field experiments conducted in vineyards and orchards revealed that several post-emergence herbicides such as amitrole can show high efficacy, especially when a pre-emergence herbicide was previously applied. In addition, agronomic practices such as mowing and ploughing can significantly reduce seed bank and almost disappear small-seeded weeds like *Conyza* spp. However, limitations on the adoption of such practices still exist. Regarding fitness studies, our results show that under non-competitive conditions, growth and seed production of the GR and GS biotypes were similar in *C. bonariensis*, *C. canadensis* and *C. albida*. The competitive ability of the GR and GS biotypes, as determined by replacement series experiments, was also similar, since the relative crowding coefficient (RCC) between the biotypes was in most cases close to one. Overall, there was no apparent fitness penalty associated to glyphosate resistance. Therefore, the GR populations are likely to persist unless effective and case-specific management strategies are adopted.

Epigenetic mechanisms and regulation of genes involved in glyphosate resistance in *Conyza canadensis*

T. Margaritopoulou[1], E. Tani[2], I.S. Travlos[3] and D. Chachalis[1]

[1] Benaki Phytopathological Institute, Laboratory of Weed Science, S Delta 8, 14561, Athens
[2]Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Breeding, Iera Odos 75, 11855, Athens
[3]Agricultural University of Athens, Department of Crop Science, Laboratory of Agronomy, Iera Odos 75, 11855, Athens

Glyphosate is the most important herbicide globally with a distinct mode of action as a competitive inhibitor of the enzyme *EPSPS;* whereas horseweed (*Conyza canadensis*) has been the most frequent weed species that developed resistance to it in various parts of the world, including Greece. Horseweed phenotypic plasticity, different phenotypes from the same genotype in response to selection pressure from glyphosate application, is documented. In a previous work, we have proposed a resistance mechanism that is not due to a point mutation at the codon 106 of EPSP synthase but likely due to a synchronized overexpression of *EPSPS* and the *ABC-transporter* genes. In the current study, it is hypothesized that the observed phenotypic alterations and differential expression of the *EPSPS* gene (involved in the glyphosate resistant pathway within populations without changes in its coding sequence) would be attributed to epigenetic changes. DNA methylation plays a pivotal role in many biological procedures such as gene expression, differentiation and cellular proliferation. Using the cutting edge technique of sodium bisulfite sequencing, variation of the expression levels of *EPSPsynthase* gene of two populations (R- vs. S-) of *Conyza canadensis* was assessed. Sodium bisulfite sequencing was used to detect epigenetic changes that occur at the C5 position of cytosine residues within CpGdi nucleotides. Our work will shed light in the naturally raised resistance of *Conyza canadensis* to glyphosate and set the bases for future development of techniques that restrict weed resistance to herbicides.

Assessment resistance to glyphosate of *Epilobium* spp. and *Conyza bonariensis* in an intensive olive grove

João Portugal[1], Calha, I M[2], Rafael De Prado[3]

1 Polytechnic Institute of Beja, 7800 Beja, Portugal
2 Agrarian and Veterinary Research Institute, 2780-155 Oeiras, Portugal.
3 University of Córdoba, 14071 Córdoba, Spain.
(jportugal@ipbeja.pt)

The Portuguese government has been building Europe´s largest irrigation project (120,000 ha) in Southern Portugal. Around 54% of this area is already occupied with intensive olive groves. The weed management adopted by the majority of the olive farmers was the application of glyphosate, at least twice a year, for weed control in the groves. Five years ago *Conyza bonariensis* (L.) Cronquist was the first case of glyphosate resistance. The second case was confirmed one year later (*C. canadensis* (L) Cronquist). In February 2014 an unusual weed population appeared in an olive grove near Beja (Portugal). The dominating weed was characterized as *Epilobium* spp (80%) and the remaining 20% was occupied by *C. bonariensis*. In order to investigate if both species were glyphosate-resistant a dose-response experiment in laboratory and a field trial were carried out. The dose-response experiment showed very low ED_{50} values for *C. bonariensis* (41.6 ae ha^{-1}) at BBCH 14-16 (4-6 leaves). The ED_{50} for *Epilobium* was lower at the seedling stage (60.7 ae ha^{-1}) as compared to rosette stage (152.7 ae ha^{-1}). These results conclude that both species are glyphosate susceptible and agree with field observations that plants at early developmental stages are more sensitive to glyphosate. The field trial was placed in a complete randomizing block with 14 treatments and every plot measured 2mx7m with tree replication. The herbicide application was made on 21st March 2014. After a 60 day glyphosate application with a field rate (1080 g a.i ha^{-1}) the visual evaluation showed 65% efficacy. However, flazasulfuron 50g a.i.ha^{-1}+ glyphosate1080g a.i.ha^{-1}, fluorxipir 300g a.i.ha^{-1}+glyphosate 1080 g a.i.$ha^{-1}$and flazasulfuron 50g a.i.ha^{-1}+ MCPA 1080g a.i.ha^{-1}+ 1080g a.i.ha^{-1} showed the best efficacy (95% to 100%). In this case, the growth stages of weeds is the factor which affects the effectiveness of glyphosate.

Keywords: *Epilobium* spp., *Conyza bonariensis*, glyphosate, ED_{50}, field trial.

Natural tolerance of *Avena sterilis* to glyphosate

Fernández-Moreno, P.T.[1]; Alcántara-de la Cruz, R.[1]; Portugal, J.[2]; De Prado, R.[1]

[1]*Department of Agricultural Chemistry and Edaphology, University of Córdoba, 14071 Córdoba, Spain*.[2]*Agrarian Superior College of Beja, Beja, Portugal.*
(pablotomas91@hotmail.es)

Avena sterilis is a weed of the Poaceae family that is widely distributed in Europe. This weed has been established in most of the olive groves in Portugal. Farmers have had some problems to control this weed using glyphosate. Since the advent of resistant *Lolium rigidum* Gaud. populations in Iberian peninsula, suspicions of resistance have been claimed in species that are difficult to control with this herbicide, for example *A. sterilis*. The objective of this study was to characterize the different mechanisms to resolve this control problem. Consequently, seeds from fields that had been exposed to glyphosate for several years ("T") were collected and compared with seeds from nearby fields, which were never exposed ("UT"). Dose-response assays showed no significant differences between populations with higher values of growth reduction 50% (GR_{50}) above 250 g ae ha^{-1}. Spray retention assays and shikimic acid accumulation were determined. At 96 hours after treatment, the values of the shikimic acid accumulation between populations were the same. As before, spray retention assays showed no significant differences. Values of absorption and translocation of ^{14}C-glyphosate were similar between populations. These results confirm that *A. sterilis* has a natural tolerance to glyphosate, due to the low values obtained in the shikimic acid accumulation, and absorption and translocation of ^{14}C-glyphosate compared with susceptible populations of other species previously studied, which had higher values.

Keywords: *A. sterilis*, tolerance, glyphosate, dose-response, mechanisms.

Characterization of resistance to glyphosate *Bidens pilosa* L. harvested in citric groves in Veracruz, Mexico.

Alcántara-de la Cruz, Ricardo[1]*; Arjona-López, Juan M.[1]; Fernández-Moreno, Pablo T[1]; Domínguez-Valenzuela, José A[2]; De Prado, Rafael A[1]

[1]University of Cordoba, Cordoba, Spain.[2]Chapingo Autonomous University, Chapingo, Mexico.
**(g12alalr@uco.es)*

The State of Veracruz, Mexico is the highest producer of citric fruits, mainly oranges (*Citrus sinensis*) and persian limes (*Citrus latifolia*). Persian lime is an important economical crop in this region, so it receives more care than other citric crops. Weed control represents one of the most important crop-care aspects, because it is made from 4 to 6 times per year. In 2010, *Leptochloa virgata* was reported as the first glyphosate resistance weed species in these crops. Recently, in 2014 has been confirmed by this investigation group. However, glyphosate remains the main chemical control tool used by farmers which has caused great changes in weed flora. Later prospections have allowed the identification of new possible cases of glyphosate resistance. *Bidens pilosa* L.is a native Asteraceae weed from Mexico with a wide distribution in tropical areas of the country. Recently this specie has been reported as a glyphosate resistance species. In this work we have carried out preliminary characterization studies of glyphosate resistance in a sensitive population (S) and two resistant populations (R1 and R2) of this species. Dose-response assays in greenhouse indicated that the R1and R2 populations were 24.8 and 2.5 times more resistant in comparison to the S population, respectively. There were no significant differences in the foliar retention of herbicide in the S and R1 populations. However, the R2 population retained 1.6 times more herbicide solution (ml g^{-1} dry weight) respect to the S and R1 populations. Absorption and translocation studies with ^{14}C-glyphosate were conducted only in S and R1 populations. Qualitative results of ^{14}C-glyphosate translocation showed poor penetration and translocation to the rest of the plant and root in R1 populations respect to S population. These early results show that *B. pilosa* has developed glyphosate resistance. This resistance could be influenced by a poor penetration and translocation of glyphosate in its action site. It is necessary to continue with characterization and description studies of the mechanisms involved in that resistance.

Keywords: *B. pilosa* populations, dose-response, ^{14}C-glyphosate, foliar retention, persian limes, resistance.

Cross-resistance to ACCase-inhibitors in *Eleusine indica*. Reactive control.

Fernández-Moreno, P.T.[1]; Alcántara-de la Cruz, R.[1]; de Portugal, J.[2]; Osuna-Ruiz, M.D.[3]; De Prado, R[1].

[1]*Department of Agricultural Chemistry and Edaphology, University of Córdoba, 14071 Córdoba, Spain*.[2]*Agrarian Superior College of Beja, Beja, Portugal.*
[3]*Agraian Research Center "Finca la Orden Valdesequera", Badajoz, Spain.*
(qe1pramr@uco.es)

E. indica is a very competitive, fertile species and can be found in many types of soils and crops such as corn, cotton, sugarcane or perennial crops (orchards and groves). *E. indica* causes serious damage to crops in Brazil and therefore is considered one of the worst weeds in the country. This study examines the cross-resistance to ACCase inhibitors (APP, CHD and PPZ) and their reactive control with other herbicide action mechanisms. Doses-response and ACCase activity showed that the R population is resistant to all ACCase family herbicides. Molecular studies on the ACCase gene reveal several mutations that are responsible for the resistance to these herbicides. The study conducted on the absorption, translocation and metabolism of ^{14}C-dichlofop-methyl showed no difference between the sensitive and resistant populations. Experiments carried out in greenhouse were conducted to determine the effectiveness of other herbicides with different action mechanisms on both R and S to ACCase herbicide populations. The GR_{50} values showed two groups of effectiveness: 1st group those whose FR (GR_{50}R/ GR_{50} S) were ≥1.5 (EPSP synthase and PS I inhibitor herbicides) and 2nd group those whose FR values were ≤1.5 (ALS, Glutamine synthase, PPO and PS II inhibitor herbicides). While the 2nd group of herbicides can be used in an integrated weed management program in rotation, the 1st group shows some danger of selection of resistance and should only be used in mixtures with the 2nd group.

Keywords: *E. indica*, GR_{50}, TRS, NTRS, reactive control.

Analysis of genetic differences in R- and S- sterile oat (*Avena sterilis* L.) populations in Greece

Xanthopoulou Aliki[1, 2], Ganopoulos Ioannis[1], Madesis Panagiotis[1], Travlos I.[3] and D. Chachalis[4]

[1]Institute of Applied Biosciences, CERTH, Thermi, Thessaloniki, 570 01, Greece
[2]Department of Genetics and Plant Breeding, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki 54 124, Greece.
[3]Agricultural University of Athens, Department of Crop Science, Laboratory of Agronomy, Iera Odos 75, 11855, Athens
[4]BenakiPhytopathological Institute, Laboratory of Weed Science, S Delta 8, 14561, Athens

Sterile oat (*Avena sterilis* L.) is the most important and frequently encountered annual grass weed infesting winter cereal fields at the time of crop maturation and has developed herbicide resistance. The main objectives in population genetics are to describe the amount of genetic variation in and among populations and to study the mechanisms of variation dynamics. The genetic differences between twelve populations from Greece were investigated using ISSR molecular markers Dose response experiments were performed in order to check their level of herbicide resistance. A total of 180 plants from 12 sterile oat populations in Greece were analyzed by three primers resulting in 28 highly reproducible ISSR bands. The analysis of molecular variance (AMOVA) with distances among individuals corrected for the dominant nature of ISSRs showed that most of the variation (64%) occurred among populations, and the remaining 36% variance was attributed to differences among individuals within populations. The high differentiation was, perhaps, due to limited gene flow (Nm <1.0) of this species. High Φ_{ST} value (0.645, $p<0.001$) corroborated AMOVA partitioning and provided significant evidence for population differentiation in sterile oat. UPGMA cluster analyses, based on Nei's genetic distance, revealed grouping pattern. Bayesian structure and principal coordinate analysis revealed that twelve populations were clustered into several groups. The estimated genetic variability may be helpful in developing management strategies to reduce the impact of *A. sterilis* and other weeds in crop fields. This study should also help us understand the potential impact of herbicide selection, imposed nearly every year for the last three to four decades, on the levels of genetic diversity within and across *A. sterilis* populations.

Evaluation of resistance to imidazolinone herbicides in susceptible and resistant sunflower hybrids

A. Kapazoglou[1], O. Roussou[1], A. Marouli[1], P. J. Terzopoulos[2], P. J. Bebeli[1]

[1]Laboratory of Plant Breeding & Biometry, Department of Crop Science, Agricultural University of Athens, Greece
[2]House of Agriculture Spirou SA, Athens, Greece
(bebeli@aua.gr)

Imidazolinone herbicides inhibit the action of the enzyme acetohydroxyacid synthase (AHAS), involved in the synthesis of essential amino acids, thus leading to plant death. A particular mutation in the catalytic subunit of AHAS (A205V) renders plants resistant to imidazolinones and has been utilized for the production of resistant sunflower hybrids (Clearfield technology). The aim of this study was a) the phenotypic evaluation of a susceptible (R1) and two Clearfield (R2, R3) sunflower hybrids at 7, 14 and 21 days after spraying with 400 and 1200μg/ml imazamox at the stage of 4-6 leaves and b) the expression analysis of three genes encoding AHAS (*HaAHAS1, HaAHAS2, HaAHAS3*) 36 hours after herbicide treatment. On the 7th day after herbicide application, hybrids began to show distinct morphological characteristics (different degrees of chlorotic spots and necrotic lesions among the different hybrids) which became completely apparent on the 21st day of herbicide application and were monitored on a scale 0-5 (0, healthy; 5, mortality). At 400 μg/ml imazamox, R1 was nearly completely necrotic (>4) whereas R2 and R3 exhibited resistance to the herbicide (<4). At 1200 μg/ml imazamox, susceptibility increased for all three hybrids (>4). The expression of *HaAHAS1*, *HaAHAS2* and *HaAHAS3* was investigated in R1, R2, and R3 with quantitative Real Time PCR. Total RNA was isolated from leaf tissue after 36 hours of application with 400 and 1200μg/ml imazamox, cDNA was synthesized and qPCR performed with specific primers for each gene. Interestingly, *HaAHAS1* and *HaAHAS2* were significantly induced after treatment with 400 and 1200μg/ml imazamox in the resistant R3 hybrid compared to R1. In conclusion, the sunflower hybrids R2 and R3 exhibit resistance to imazamox at a dose of 400μg/ml and induction of the *HaAHAS1* and *HaAHAS2* genes in the resistant R3 hybrid suggests an association of increased expression with resistance to the herbicide.

3rd SESSION: NON CHEMICAL METHODS / INVASIVE WEEDS

Non chemical weed control methods and IWM

M.M. Riemens

Wageningen UR, Wageningen, The Netherlands.
(*marleen.riemens@wur.nl****)***

Current agricultural production systems in Europe can be characterized by the large amounts of inputs and high outputs (Hersperger & Bürgi, 2009). One of the factors that made the intensification of agriculture possible, was the introduction of herbicides. This can be illustrated with a study by Oerke et al (2006), who estimated that the potential yield loss that can be ascribed to weed competition is 34%, while the actual yield loss is around 10% in Europe. This suggests that the current cropping systems are highly effective in weed control. However, the strong dependency of our agricultural systems on chemical weed control is not sustainable. The use of a single control measure will result in herbicide resistance and a number of difficult to control weed species (Bastiaans et al., 2015). Already 240 weed species have been documented as resistant to herbicides worldwide, and for 22 out of the 25 known groups of herbicide chemistries (Heap, 2015). A diverse weed management system is an important prerequisite for the sustainability of our agricultural systems and global food security in the future. The use of Integrated Weed Management (IWM) strategies applied in a crop rotation context contributes to an increased diversification of the systems. Instead of simply replacing herbicides by other direct control techniques, integrated weed management focuses on the management of weed populations at a time scale extending the current growth season (Kruidhof *et al*., 2008). IWM strategies are based on three key principles (Bastiaans et al 2008): 1) manipulation of the competitive relationship between crop and weed, 2) a reduced recruitment from the soil seed bank and 3) a gradual depletion of the soil seed bank. Several effective strategies were developed within the research community in general and the EWRS working group “physical and mechanical weed control” in specific and most recent developments will be highlighted. Despite the progress that has been made by the research community there is evidence that science alone will not change weed management practices. Many farmers have not embraced IWM practices despite their proven ability to mitigate weed problems and leading to sustainable weed management. To get a better understanding of farmer decision making, a mental model of weed management was developed for Dutch farmers. A mental model is a complex web of beliefs, that affect how an individual defines and reacts to a problem, gathers and assesses information, and makes decisions. The model shows that the knowledge, beliefs, perceptions and attitudes that underlie weed management activities of farmers are influenced by their previous experience, contact with peers, and

perceived risk of different management options and to a very little extent by scientific results. This emphasizes the importance to include farmers and their needs in an early stage of the development of IWM strategies.

Biological weed management in Assam

A.Devee, M. Borah, D.K. Saikia, P. Dutta and K.C. Pujari

Assam Agricultural University, Jorhat-13, Assam, India

Assam is a state of India located in the north-eastern region, south of the eastern Himalayas, comprises with river valleys of Brahmaputra, Barak and Karbi Anglong hills. Total area of Assam is 78,438 km² including River basin of Brahmaputra, Barak and their tributaries. The total area under different categories of wetland in Assam is about 1012.32 km^2. Of the total wetlands, 1367 numbers of inland wetlands suffer due to the problem of invasion by aquatic weeds. The valley of the river Brahmaputra with its innumerable fresh water lakes contained various fishes, fresh water dolphin, dugong, Indian one-horned rhino, crocodile, the winter monitor lizard and few species of turtles. All these creatures are either extinct or highly endangered at present with the progressive destruction of these wetland systems which was started with the arrival of the water hyacinth from Central America more than a century ago. The other most dominant weeds are Nymphoides spp., *Trapa Bisinosa, Salvinia spp., Azolla pinnata, Colocasia antiquorum, Marsilea quadrifolia*, Hygrophila (*Hygrophila polysperma*,) etc. Extensive growth of these weeds can cut out sun light from the micro flora and also produces faster eutrophication by slowing down water current and depositing debris at the bottom and also blocks the irrigation channels. Assam belongs to high humid tropical climate with high temperate (summer max 35–38 °C and winter min. 6–8 °C), heavy rainfall (Average 2818 mm/year) and high levels of humidity (average 79% - 85%). This type of climate favours the fast growth of many weeds. Tea and sugarcane are two major important cash crops of Assam and *Mikania micrantha* is the major cause of reduction of yield of these crops. In case of tea 10-50 % reduction of productivity occurs due to *M. micrantha*. Besides reducing the yield, weeds also produce the adverse effects by restrict growth of crops by covering the whole plant, serve as alternate host for many pests, and water flow in the drains. *Mikania micrantha*, in Assam alone causes a loss of 18.90 to 41.8% of tea productivity. Presently, infestation of

Mikania posing serious threat to plantation crop, agricultural field, natural forests in Assam due to its rapid growth rate, large reproductive outputs, efficient dispersal capabilities and tolerance to broad range of environmental conditions. Mechanical and chemical methods of management are undesirable because of inherent problems associated with each method. In view of this, bio intensive management strategies are the only alternative and the urgent need of the hour to combat the menace. The study on 'Biological weed management in Assam' is undertaken to focus the importance of different bioagents in weed management. The study was done on the basis of primary (on field experiments) and secondary (from literature) information. The release of exotic natural enemies, mottled water hyacinth weevil, *Neochetina eichhorniae* and *N. bruchi* gave a control of 90-95% of water hyacinth in 1000 hactares of water body after 21 months of initial release of weevils in Jorhat and Sivsagar district of Assam. Hygrophila (*Hygrophila polysperma* (Roxb.)) is another important aquatic weeds of Assam and from 34 different survey cites, several insects were collected, including two caterpillars (Lepidoptera; families Nymphalidae and Noctuidae) that defoliate emerged plants; one aquatic leaf cutting caterpillar (Lepidoptera, family Crambidae) and a leaf mining beetle (Coleoptera, family Buprestidae). Introduction of *Puccinia spegazzinii*, a rust fungus in Mikania control in limited tea ecosystem of Assam was already started and found effective results. This Puccinia strain was tested against 55 non-target species, including crops, and proved to be totally specific against Mikania, to which it is highly damaging, resulting in leaf, petiole and stem cankering and death of the whole plant. As the different herbicides have various adverse effects, these bio agents have tremendous potential in management of weeds. The proper identification and validation of these organisms may help in development of different IPM strategies in reducing the herbicides loads in the environment in near future.

A first approach of chemical control of silverleaf nightshade (*Solanum elaeagnifolium*) with the herbicide tembotrione

T.K. Gitsopoulos[1], C.A. Damalas[2], I. Georgoulas[1]

[1]Hellenic Agricultural Organization-Demeter, Institute of Plant Breeding and Phytogenetic Resources, 57001 Thermi, Greece
[2]Department of Agricultural Development, Democritus University of Thrace,
68200 Orestiada, Greece
Email: gitsopoulos@yahoo.gr

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) originating from the Americas, is considered to be one of the most invasive plants worldwide and a major weed problem in many temperate agricultural areas. This weed can be difficult to control both mechanically and chemically due to the extensive root system and the production of highly viable seeds that results in the establishment of extensive seed banks. Field experiments were conducted at Thermi, Greece to evaluate within-season chemical control of established silverleaf nightshade plants with the HPPD-inhibitor tembotrione. Experiments were established in a naturally infested field after chickpea harvest and shallow soil tillage with rotavator. Herbicide treatments included single applications of tembotrione at 100 and 150 g aiha^{-1}and split applications of tembotrione at 100 plus 50 and at 75 plus 75 g aiha^{-1}with 5-day interval. Herbicide applications were performed with an air-pressurized hand-field plot sprayer, with a 2.4 m wide boom fitted with six 11002 Teejet twin fan nozzles, calibrated to deliver 400 L ha^{-1}. Non-treated control was included for comparison. Herbicides were applied when the majority of plants were 15 cm tall. Plants treated with tembotrione showed the first injury symptoms four to five days after treatment. Herbicide symptoms included typical bleaching of the new leaves that eventually turned necrotic. Efficacy was assessed by determining height and fresh weight of plants exhibiting symptoms of herbicide injury (necrotic parts) 30 days after treatment. Retardation and inhibition of growth was recorded on silverleaf nightshade treated with tembotrione plants. None of these plants reached the fruit stage. Based on fresh weight assessment, the chemical treatments resulted in 74 to 80% silverleaf nightshade shoot control.

De novo transcriptome analysis of the weed *Solanum elaeagnifolium* to elucidate the molecular pathways that contribute to stress response

Tsaballa A.[1]; Makris A.M.[1]; Argiriou A.[1]

[1] Institute of Applied Biosciences, Centre for Research and Technology Hellas, Thermi Thessaloniki, Greece
(afroditi.tsampalla@gmail.com)

Silverleaf nightshade (*Solanum elaeagnifolium)* is a perennial weed that belongs to the *Solanaceae* family. Although native of America, it is considered an alien, highly invasive and well-established species in the Mediterranean basin. Furthermore, in many countries it is a quarantine pest. *S. elaeagnifolium* can survive and develop in various environments, even in the most deteriorated soils and it is especially resistant to drought. One of its most typical and vital characteristics is the ability to propagate both sexually and asexually by underground growing plant parts and therefore very difficult to be managed by the use of herbicides. The plant can host several plant viruses and its fruits are toxic to many livestock animals. Its capability to withstand the unfavorable - for other plants - environmental conditions and spread in non favorable places has prompted us to study the plant's gene networks in order to identify possible molecular pathways that facilitate *S. elaeangnifolium*'s adaptation to stresses. Here we report the *de novo* sequencing, assembly and analysis of leaf and flower *S. elaeagnifolium* transcriptome, using next-generation Illumina sequencing. 51,234,362 clean reads were produced that were assembled in 75,618 Unigenes of 1,082bp mean length. Since one of the basic mechanisms by which plants respond to environmental stress is the synthesis of specific secondary metabolites that protect the plant from herbivores and microorganisms, or serve as signaling molecules, we emphasized on the study of secondary metabolites such as terpenes. Several *S. elaeagnifolium* transcripts were identified by bioinformatics analysis as putative *Terpene Synthase* (*TPS*) genes. A phylogenetic tree was constructed and it was found that *S. elaeagnifolium* deduced aminoacid sequences are placed in TPS-a, TPS-b, TPS-c, TPS-e/f, TPS-g clades of TPS proteins. Gene cloning and expression analysis of selected *TPS* were performed. A leaves wounding experiment has proved the significant transcriptomic induction of a putative sesquiterpene *TPS* gene. Further analysis and study of more *TPS* and other genes and molecular pathways putatively involved in the plant's response to environmental stimulus will pave the way for the elucidation of the mechanisms that lie beneath the complex features of this unique Solanum weed species.

Integrated weed management (IWM) in Iranian potato fields and role of herbicide optimization as a new challenge

Alebrahim, M. T.[1], **Majd, R.**[2] **and Kudsk, P.**[3]

[1]*University of Mohaghegh Ardabili, Ardabil, IRAN*
[2]*University of Mohaghegh Ardabili, Ardabil, IRAN*
[3]*Aarhus University, Slagelse, Denmark*
(Corresponding author: m_ebrahim@uma.ac.ir)

Increasing agricultural production during the 20th century has resulted in increased use of inputs and intensification of agricultural activities in order to control weeds, soil erosion, and pollution by chemicals and herbicide-resistant weeds. For accessing this important goal; Iranian researchers and farmers were using different methods for weed managing in potato fields including: rotation, fertilizer types and techniques of application, live mulches, black and transparent polyethylene sheet, cultivation, herbicides and bioherbicides. Regarding to low number of herbicides which are registered in Iran for potato (paraquat and metribuzin) and their deficiency for successful control of weeds during growing season, researchers persuade to increase number of herbicides and in recent years, weed scientist have paid more attention towards integrated weed management and application of reduced rate of herbicides to prevent environmental pollution. Ethalfluralin, trifluralin, pendimethalin, rimsulfuron, EPTC and oxadiargyl are six new herbicides for Iranian potato fields and have different advantages and disadvantages up to their application in discrete geographic locations, cultivars and distinct dominant noxious weeds. Currently herbicide optimization focus on finding reduced doses, best growth stage, spraying time and adjuvant.

Keywords: IWM, potato, Iran, herbicide optimization.

4th SESSION: IWM AND HERBICIDE USE: OPTIMIZING PERFORMANCE AND MINIMIZING NON-TARGET EFFECTS / ENVIRONMENTAL ISSUES

Inspection of spraying equipment in Greece

M. Vlachogiannis

TEI of Thessaly, Department of Mechanical Engineering, Larisa
(mvlach@teilar.gr)

The EcoPest project was a pilot-scale demonstration project implemented in the Kopaida region, about 70km north-west of Athens. Among the requirements of the SUD were the actions adopted for the calibration and maintenance of spraying equipment in order to comply with the European standards. This is the first major attempt at a large regional scale, to fulfill the requirements for complying with the SUD carrying out spraying equipment inspections, calibration, regulation and maintenance in order to ensure machinery optimum operation. The objective of this presentation is, apart from the legal need to the importance of proper operation of spraying machinery addresses issues related to efficacy as well as minimization of pesticide quantity applied. It is known that spray drift is one of the most important routes for the pollution of surface waters by pesticides, contamination of adjacent crops, and exposure of wildlife, workers, bystanders and the agricultural population to pesticides. Considering that some of most important factors affecting spray drift are the pesticide droplet size –mostly dependent from the type of spraying nozzles used - and the sprayer's operation conditions and parameters (spraying pressure, tractor speed, nozzle direction/angle etc) it becomes even more evident that the spraying machinery status is a factor of major importance to the promotion of both agro-environmental safety as well as public health.

Safe use of herbicides in maize seed crop

Brankov M., Simić, M., Dragičević, V., Spasojević I.

Maize Research Institute "Zemun Polje", Belgrade, Serbia
(mbrankov@mrizp.rs)

In some crops, like maize seed, herbicide application can negatively affect crop plants. Through breeding, maize plants come in homozygote stage, which lead to slower germination, lower growth and higher sensitivity to various factors. Those properties allow higher weed infestation in field, so herbicide application is one of the most important practices of IWM. On the other hand, if maize plant are stressed, by e. g. meteorological conditions, herbicide application can cause plant damage, or in worst case yield loss. In Serbia, herbicides are not registered for use in maize inbred lines. So, safe herbicide application requires maize lines sensitivity testing for minimum two years, similar like in registration. Five maize lines from different heterotic groups were tested in three year experiment on the experimental field of the Maize Research Institute Zemun Polje, Belgrade, Serbia. Herbicides were applied when maize developed 5-6 leaves (15-16 stage BBCH) in recommended dose for application in hybrid maize and in double dose. Two groups of herbicides were applied: triketons - mesotrione 120 and 240 g a. i. ha^{-1} and topramezone 67.2 and 134.4 g a. i. ha^{-1}; and sulfonylureas - rimsulfuron 15 and 30 g a. i. ha^{-1} and foramsulfuron 45 and 90 g a. i. ha^{-1}. Visual plant damages (according to EWRC scale) were estimated in period of 2-3 weeks after herbicides application, as well as 2-3 weeks after first estimation. Plant fresh mass was measured also two times: first in the same time when first visual damages were estimated, and in flowering stage. At the end of vegetation period grain yield was estimated. Obtained data was statistically processed by ANOVA and differences between means were tested by LSD test. Sulfonylurea herbicides caused higher plant damages compared to triketons. Both doses of foramsulfuron caused moderate to tolerable damages to two maize lines (lines early maturity group, L1 and L2). Rimsulfuron applied in both doses induced moderate damages to those two lines. In second estimation similar plant damages were recorded for sulfonylureas. Other lines had minor injuries from sulfonylureas. Injury symptoms were followed with decrease of fresh mass, especially 2-3 weeks after herbicide application. Double dose of foramsulfuron in higher percentage decreased fresh mass on three maize lines (L1, L2 and L3). Similar results were observed in maize flowering, where fresh mass was decreased when sulfonylureas were applied. Sulfonylureas decreased grain yield in lines all lines except L4. On the other hand, triketons induced slightly injuries according to first evaluating, while no injury symptoms were recorded during second evaluation. Mesotrione and topramezone did not influence on fresh mass and grain yield of maize lines. According to obtained data, triketons herbicides can be safely applied in maize inbred lines crops, while for sulfonylurea herbicides attention is required.

Four years validation of decision support optimising herbicide dose in cereals under Spanish conditions.

Montull JM[1], Soenderskov M[3], Rydahl P[3], Boejer OM[3], Taberner A[1&2]

[1]Research Group of Weed Science and Plant Ecology. UdL. Av. Rovira Roure 191
[2]Plant Health Service. Generalitat de Cataluña Av. Rovira Roure 191. 25198 Lleida. Spain
[3]Dept. of Agroecology. Aarhus University. Forsoegsvej 1. 4200 Slagelse. Denmark
ataberner@gencat.cat

The Danish decision support system Crop Protection Online (CPO) optimises herbicide weed control. CPO recommends specific herbicide solutions to achieve a required level of control. The aim is to apply herbicides as little as possible but as much as necessary. CPOWeeds is a version of CPO adjusted to conditions in North-eastern Spain. The predicted efficacies and the yield obtained with CPOWeeds were validated in winter cereal field trials from 2010 to 2013. All CPOWeeds treatments were related to the efficacies obtained with standard herbicide treatments decided upon by local advisors. The predictions from CPOWeeds were compared to the actually achieved efficacies in the field trials for the nine weed species at different developmental stages and for 84.2% of the comparisons the obtained efficacies were equal to or higher than predicted. The average difference between predicted and observed efficacies was 2.35 percentage points. Yield was measured in three trials and the recommendations from CPOWeeds were maintaining yield. There were two situations where CPOWeeds were performing suboptimal. One is in the early weed growth stages, as the model is not yet prepared to account for water stress on root action herbicides applied at 10-11 BBCH. The second situation was in fields with a prior unidentified population of resistant *Alopecurus myosuroides*. For key species in winter cereals in Spain, such as *Avena sterilis, Lolium rigidum* and *Papaver rhoeas*, CPOWeeds achieved a satisfactory control level. It was concluded that the use of CPOWeeds allowed optimisation of the herbicide application with a very high robustness. The recommendations were satisfactorily for the conditions of the Northeast of Spain and have the potential to decrease the amount of applied herbicides by at least 30%. Therefore, it can be an important tool in Integrated Weed Management.

Keywords: weed control; optimised herbicide dose; decision support system; factor adjusted dose.

Reduced rates of herbicides to control *Galium aparine, Sinapis arvensis* and *Avena sterilis* in late-sown durum wheat (*Triticum durum* L.)

Karkanis, A.[1], Bilalis, D.[2], Efthimiadou A.[3], Travlos, I.[2], Chachalis D.[4], Katsenios, N.[5]

[1]University of Thessaly, Volos, Greece
[2]Agricultural University of Athens, Athens, Greece
[3]New York College, Athens, Greece
[4]Benaki Phytopathological Insitute
[5]Soil Science Institute of Athens, Athens, Greece
(anekark80@yahoo.gr)

In recent years, there has been increased interest from farmers to implement integrated weed management (IWM) programs. The main objective of these programs is to reduce the amount of herbicides applied to crops. This goal can only be achieved if combined with appropriate culture practices. Sowing date and plant density are crucial for IWM programs in wheat crops. As wheat growers begin to plant earlier in the growing season at reduced seeding rates, weed management is more dependent on herbicide rates for adequate weed control. Wild mustard (*Sinapis arvensis* L.), cleavers (*Galium aparine* L.) and wild oat (*Avena sterilis* L.) are among the most troublesome weeds in wheat and other winter cereals. Therefore, the aim of this study was to determine the efficacy of post-emergence herbicides applied at the recommended and reduced rates on above weeds in late planted winter wheat. A field experiment was conducted in Domokos region in Central Greece in 2013-2014. Durum wheat (*Triticum durum* cv. Quadrato) was sown in the middle of December, at a rate of 280 kg ha^{-1}. A randomized complete block design was employed with four replicates per treatment. The plot size was 3 x 3.5 m. The herbicides treatments included untreated control, weedy control, pinoxaden+2.4D+florasulam at three doses (0,90 X, X (minimum recommended dose) and MX (full recommended dose)), and mesosulfuron-methyl+iodosulfuron-methyl-sodium at three doses (0,90 X, X (minimum recommended dose) and FX (full recommended dose)). The recommended doses of pinoxaden, 2.4 D +florasulam and mesosulfuron-methyl+iodosulfuron-methyl-sodium were 40-45 g a.i ha^{-1}, 180-240 g a.i ha^{-1} + 3.75-5 a.i ha^{-1}, 6-7.5 a.i. ha^{-1} and 6-7.5 g a.i. ha^{-1}, respectively. The herbicides were applied at wheat tillering. Weed control efficacy was determined, while crop parameters such as plant height, number of tillers, ear length and seed yield were also measured. Analysis of variance was conducted for all data and differences between means were separated using Fisher's Least Significant Difference (*LSD*) test at $P < 0.05$. Our results revealed that the selected herbicides (pinoxaden+2.4D+florasulam and mesosulfuron-methyl+iodosulfuron-methyl-sodium) could effectively control *A. sterilis.* and *G. aparine* when its dose was reduced by 10%. The higher efficacy was obtained with the maximum dose. It was also found that that 2.4D+florasulam acted better against *S. arvensis* than mesosulfuron-methyl+iodosulfuron-methyl-sodium. Also, 2.4D+ florasulam at 5 a.i. ha^{-1} gave the best control of *S.*

arvensis. Furthermore, all herbicide treatments improved wheat growth and seed yield compared to the untreated control. Finally, our results show that the weed control may be achieved by the application of herbicides at reduced doses in late sown wheat.

Effects of glyphosate application on arbuscular mycorrhizal fungi of the succeeding crop

A. Gavalaki[1], N. Koustas[1], A. Antoniadis[1], I. Pantelis[1], I.S. Travlos[1], D.J. Bilalis[1], Y. Papatheohari[1], I. Dede[1] and M. Druille[2]

[1] Faculty of Crop Science, Agricultural University of Athens, Athens, Greece
[2] University of Buenos Aires, Institute for Agricultural Plant Physiology and Ecology (IFEVA), Buenos Aires, Argentin

Glyphosate is a systemic non-selective herbicide, one of the most widely used in the world. Its action is based on inhibition of the enzyme involved in the synthesis of aromatic amino acids tyrosine, tryptophan and phenylalanine. However, its effects on non-target organisms, such as arbuscular mycorrhizal fungi (AMF), are rather unclear. AMF colonizes the roots of the majority of terrestrial plants, improving their growth and performance, by improving water relations, uptake of nutrients and protection against several pathogens. The aim of the present study was to evaluate the potential effects of glyphosate application on mycorrhizal colonization and plant growth of the succeeding crops. *Vicia sativa*, *Lathyrus sativus* and *Lupinus albus* were selected, since legumes are characterized by greater mycorrhizal dependency than other crops. Glyphosate was applied in bare soil at recommended and double rate, while there were also some untreated pots. Two weeks after glyphosate application, seeds of the above-mentioned species were sown. Plants were harvested 45 days after sowing and AMF root colonization was properly assessed. Our hypothesis was that herbicide application may affect the AMF colonization of the succeeding crops. The results of the present study indicated that in some cases there was a delay of the plant growth in the pots sprayed with double the recommended rate of glyphosate compared with the other pots. Regarding the total root colonization this was ranged from 77 to 86 % for all the untreated plants. However, for *V. sativa*, previous spraying with glyphosate resulted to a reduction of AMF root colonization by 31 and 64 % for the recommended and double rate, respectively. In the cases of *L. sativus* and *L. albus*, the relative reduction was significant and ranged from 55 to 57%, regardless of the rate. Conclusively, it could be said that the common practice of glyphosate use and especially in very high rates before the establishment of new crops may damage AMF of the next crop. These indications should be confirmed in a field level and further studies should be conducted under different soil and climatic conditions.

Assessment of pollution levels and toxicity of environmental samples collected from the monitoring network developed in the frame of EcoPest LIFE+ project

K. Kyriakopoulou, E. Karasali, V. Kati, P. Sarli and K. Machera*

Department of Pesticides Control and Phytopharmacy,
Benaki Phytopathological Institute Kifissia, Greece
*(*corresponding author, K.Machera@bpi.gr)*

EcoPest was a LIFE+ project lasted from January 2009 to March 2012. The main objective of the project was the development of an integrated strategy for the adaptation and application of the principles for the sustainable use of pesticides in a vulnerable ecosystem. EcoPest was applied at a pilot scale, in an area of 900 ha agricultural land with arable crops (cotton, maize, plum tomatoes) at Viotikos Kiffissos river basin adjacent to lake Yliki. A distinct action of the project dealt with the environmental monitoring of the pilot area providing information about pollutant levels in water and soil related with agricultural activities. For this purpose an environmental monitoring network has been established taking into consideration the hydrological, hydrogeological and morphological characteristics of the pilot area, as well as the type of crops. Water and soil environmental samples were collected at specific time periods during 2009, 2010 and 2011 and analysed for the determination of pesticide residues according to certified methods with gas and liquid chromatography. Furthermore, direct toxicity assessment of the samples was performed on aquatic and soil indicator organisms (bacterium *Vibrio fishery*, green alga *Selenastrum capricornutum,* crustacean *Daphnia magna* and earthworm *Eisenia fetida*). The results of analytical measurements and bioassays were compared between 2009 (baseline situation) and 2010 and 2011 (1^{st} and 2^{nd} year of Low Crop Management (LCM) System implementation respectively). In 2010 and 2011 significant reduction (up to 70%) was observed in mean pesticide concentrations detected in water samples collected from wells. This reduction mainly concerned herbicides, while a significant increase was observed in the concentrations of insecticides during 2010 when compared with 2009. This increase was predicted and justified due to an outbreak of cotton ball warm, in the pilot area during 2010. Furthermore, significant reduction of the mean concentrations of pesticide residues has been observed in soil samples in 2010 and 2011. Significant reduction was observed in the toxicity of the tested samples on green alga *Selenastrum capricornutum* in 2010 and 2011 compared to 2009, mainly attributed to the reduction of herbicide concentration. On the contrary, a slight increase was observed in the toxicity on crustacean *Daphnia magna* probably due to the increase of the mean concentration of insecticide residues. Finally, no significant toxicity was observed in any of the tested soil samples on earthworm *Eisenia fetida*. From the results of the present study the direct correlation of the environmental quality and pollutant levels with the applied field practices was demonstrated.

Exploring the beneficial role of weeds in olive groves for biodiversity and soil quality

G. Michalopoulos, **SAGE10 team & OLIVECLIMA team**

Weeds have been among the principal players in two LIFE projects established in olive groves in western Peloponnese and northern Crete since 2010. In the first project SAGE10 (2010-2014) weeds have been used as indicators of biodiversity, alongside with assessments of some soil species and a few other groups of organisms representative of local flora and fauna. The objective of SAGE10 has been to develop a method to objectively prioritize the environmental aspects of olive growing per single olive grove. The scope of the project was 600 olive groves split equally in three areas, two in Crete and one in w. Peloponnese. Prioritization of the aspects was against the impacts forecasted according to the values of the parameters that influence them, e.g. how moderately salinized water might affect weed species in an area. Parameters examined were both inherent (environmental) and man-driven such as harshness of farmers practices, e.g frequency / intensity of herbicide treatments. Results are still being worked on, but seem promising in the sense of verification of the forecasts of the method. The second project, OLIVECLIMA established in 2012 on 120 of the above 600 olive groves deals with the winter weeds as CO2 traps, that under a regime of zero tillage can contribute to long term storage of carbon in the soil, as a measure for climate change mitigation. Since this long term storage is in the form of increased organic carbon content of top soil (0-40 cm) this is considered adequately beneficial for the olive crop as well as for the soil quality. So, this practice can be considered also an ‘adaptation’ technique for the threatened climate change. Results are expected after the termination of the project in 2017.

The development of an algorithm for identification and monitoring of highly vulnerable zones for pesticides contamination to ground water.

S. Vizantinopoulos, G. Arampatzis, A. Ilias, S. I. Vrouchakis, P. Dalambakis, S.Stathaki, E.Hatzigiannakis and A. Panagopoulos

Hellenic Agricultural Organisation-Land Reclamation Institute, 57400, Sindos, Greece
e-mail: sp.vizant@nagref.gr

The EC-regulation 1107/2009 concerning the placing of plant protection products (PPPs) on the market requires implementation of monitoring studies in cases where an active substance or its metabolites during risk assessment procedure results to a possible contamination of groundwater resources. This shows the importance of monitoring studies and consequently the good design of them, especially in investigating residues of pesticides in groundwater studies. Compilation of monitoring networks is a time-consuming, laborious and very responsible task that requires knowledge and expertise. In the literature exists much work on the principles that one should consider in setting up a monitoring network to ensure accuracy, reliability and representativeness of collected data. However so far, the identification and monitoring of highly vulnerable zones are addressed essentially on the basis of principles to be followed and not on a hierarchized automated selection procedure. To face this a methodology**,** based on an algorithm**,** was developed to support the selection procedure of monitoring points opting for objective and scientifically justified monitoring networks compilation, through an easy to implement multi-criteria analysis. The algorithm secures that selection of monitoring points follows a common unbiased approach. This algorithm considers a set of criteria each of which is assigned a specific weighting factor and a class, in order to compute a rank for each candidate monitoring point. The algorithm is based on parameters related to the properties of the active substance influencing its leaching process, soil**,** hydrological and geo-morphological data for each sampling point, agronomic characteristics of the system, kind of crop-application of the PPP and on technical specifications& accessibility conditions for each sampling point. Implementation of the selection algorithm in the framework of individual studies that focused on PPPs residues in groundwater at different basins showed that monitoring points selected are in good agreement with those appointed through a conventional procedure performed on the basis of local expertise judgment.

POSTER PRESENTATIONS

Morphological and molecular characterization of 15 *Echinochloa* biotypes based on the intergenic spacer of *psbK- psbI*genes of cpDNA

G. Tsoktouridis[1], K. Dhima[2], I. Vasilakoglou[3] and T.K. Gitsopoulos[4]

[1,4]Hellenic Agricultural Organization-Demeter, Institute of Plant Breeding and Phytogenetic Resources, Thermi, Greece
[2]Technological Educational Institute of Thessaloniki, Dept. of Agricultural Technology, Sindos, Greece
[3]Technological Educational Institute of Thessaly, Dept. of Agricultural Technology, Larisa, Greece
Email:gtsok1@yahoo.co.uk

The relationship among 15 *Echinochloa* biotypes was studied, by applying Polymerase Chain Reaction (PCR) in chloroplast DNA and analyzing the main morphological traits of these plants. These barnyardgrass biotypes were collected from various rice fields, which were all survived after herbicide application. The determination of *Echinochloa* species presented in each rice field is essential for their effective control, because of the existingvariability, regardingtheir susceptibility on rice herbicides. Morphological data based on the phenotypic differences of inflorescence (structure of panicle, colour of glumes and size of awns) taxonomize these plants into three main species of *E. crus-galli*, *E. phyllopogon* and *E. erecta*. In addition, molecular analysis of the intergenic spacer of *atp*F-*atp*H, *psb*K- *psb*I*trn*H-*psb*A gene regions and part of the *rpo*C1 gene of chloroplast DNA will elucidate the genetic differences among these biotypes. In the current study, only the nucleotide analysis of the intergenic spacer of *psb*K- *psb*I genes is demonstrated. A 439 bp DNA fragment of the intergenic spacer of *psb*K- *psb*I genes was amplified and sequenced. Nucleotide differences were identified in 7 loci, which characterize and group the 15 *Echinochloa*biotypes. A phylogenetic tree was constructed showing the genetic relatedness of these barnyardgrass biotypes.

Benzobicyclon: a new herbicide for late watergrass (*Echinochloa phyllopogon*) control under flooded conditions

T.K. Gitsopoulos[1], C.A. Damalas[2], I. Georgoulas[1]

[1]Hellenic Agricultural Organization-Demeter, Institute of Plant Breeding and Phytogenetic Resources, 57001 Thermi, Greece
[2]Department of Agricultural Development, Democritus University of Thrace,
68200 Orestiada, Greece
Email: gitsopoulos@yahoo.gr

Benzobicyclon is a novel HPPD inhibiting herbicide currently used in rice production systems in Japan. In previous studies, benzobicyclon showed activity at rates of 200-300 g aiha^{-1} when applied from pre-emergence to early post emergence for the control of annual grasses, sedges and broadleaf weeds in paddy rice fields, exhibiting excellent selectivity to transplanted/direct-seeded rice. In this study, two glasshouse experiments were conducted to evaluate the efficacy of benzobicyclon applied pre-emergence on late watergrass under flooding. Late watergrass seeds were seeded in 4-L plastic pots which were flooded at a depthof 5 cm the same day to establish permanent anaerobic conditions. Thefollowing day, additional water was added to the pots, where necessary, to maintain a uniform water depth of 5 cm in all pots and then benzobicyclon was applied to the flooded surface of each pot in a suspension concentrate (SC) formulation (GWN-10235, 40% benzobicyclon) at rates of 75, 150, 300 and 600 g aiha^{-1}.In pots treated with benzobicyclon, the emerged coleoptiles of late watergrass appeared typically bleached. Most plants treated with 75 g aiha^{-1}, although bleached, continued to develop till the 2nd leaf growth stage and finally became necrotic. Late watergrass plants treated with all the other rates got bleached and after reaching the 1st leaf growth stage became necrotic. Four weeks after herbicide application, benzobicyclon showed excellent control of late watergrass. Based on a visual weed control scale (from 0 to 100%), control of late watergrass was >90% at 75 g ai/ ha^{-1} and almost complete (98-100%) at the higher rates. Overall, this new herbicide can be a useful tool under flooded conditionsfor the control of late watergrassand particularly of resistant biotypes.

Mechanism of resistance to PPO and ALS inhibitors in wild poinsettia (*Euphorbia heterophylla* L.) biotypes from Brazil

Trezzi, M.M.[1]; Pacheco, V. [1];Alcantara de La Cruz, R. [2]; Fernandez-Moreno, P.T. [2]; Pagnoncelli Jr., F. [1]; Alcántara, E. [2]; De Prado, R.[2].

[1]Universidade Tecnológica Federal do Paraná,Pato Branco, Brazil.[2]Universidad de Córdoba, España.
(mailto:aminacorinna@yahoo.frvacy_vp@hotmail.com)

Wild poinsettia (*E*uphorbia *heterophylla* L.) is an annual weed species originated in tropical America and distributed in most of the tropical and subtropical areas of the world. The plants are very competitive and are particularly troublesome in soybeans, cowpeas, maize and sugarcane. Wild poinsettia biotypes suspected to have multiple resistance to PPO and ALS inhibitors were found in soybean areas from Southwest region of Paraná State, Brazil, and resistance was later confirmed by dose-response curves. The research group of Action of Pesticides on the Environment from Universidad de Córdoba, Spain, is investigating the mechanism of resistance to acetolactate synthase (ALS) and protoporphyrinogen oxidase (PPO) inhibitors in *E*. *heterophylla* biotypes. Preliminary research evaluates if the absorption and translocation of PPO inhibitors are related to resistance. Three assays were carried out to study the absorption and translocation of the radiolabeled fomesafen (PPO inhibitor) applied in leaves or roots. The experiments were conducted in a completely randomized design arranged in a factorial scheme. The first assay was arranged in a 2 x 2 x 5 factorial scheme, composed by biotypes (R and S), number of drops on leaf surface (1 and 5 drops)and five evaluation periods (1, 6, 12, 24 and 48 hours after treatment - HAT). Seeds were germinated in petri dishes and seedlings were transplanted to pots with sand + pet moss substratein greenhouse conditions until plants have four true leaves. ^{14}C-fomesafenwas mixed with commercial formulated fomesafen and surfactant to prepare a solution with a specific activity of 0.834 kBq/mL and a rate of 250 g ha^{-1}. The fourth leave of each plant was treated with one or five drops of the radiolabeled solution. In the second assay, seedlings were transferred to a hydroponic system in small pots containing nutrient solution. After two days, ^{14}C-fomesafen (1.668kBq/mL) + commercial fomesafen + surfactant was added to the nutrient solution and evaluations performed at 12, 24 and 48 HAT, in R and S biotypes. The third assay used the same methodology described in the second assay and compared absorption and translocation of fomesafen with or without surfactant at 24 HAT, in R and S biotypes. The unabsorbed ^{14}C-fomesafen in the treated leaf (exp. 1) and roots (exp. 2 and 3) was removed with 2 mL of a70:30 v/v water: methanol solution and then the plants were herborized. Cyclone Plus storage phosphor systemwas used to obtain autoradiographs. Preliminary results indicate that wild poinsettia leaves absorb about 58% of the applied ^{14}C-fomesafen within 48 HAT. Autoradiographs show that very little ^{14}C-fomesafen moves inside leaves or is translocated to other plant parts. Furthermore, fomesafen rapidly moved from

roots to shoots, mainly when surfactant was present.Absorption and movement of fomesafen was similar in R and Sbiotypes, although S biotype showed phytotoxicity. This suggests that other mechanisms are responsible for the differences in resistance.

Keywords: ^{14}C-fomesafen, absorption, translocation, shoots, roots.

Multiple-resistance to oxyfluorfen in glyphosate resistant grass weed species.

Fernández-Moreno, Pablo T[1]; Alcántara-de la Cruz, Ricardo[1]*; Trezzi, Michelangelo M[2]; De Prado, Rafael A[1]

[1]University of Cordoba, Cordoba, Spain.[2]UniversidadeTecnológica Federal Do Paraná, Pato Branco, Brazil.
**(g12alar@uco.es)*

In Spain, numerous biotypes of the genus *Lolium spp*. affect the management of perennial crops of olive groves, vineyards and citrus groves. The use of herbicides to control weeds is threatened by the emergence of resistant biotypes. This situation causes concern to Spanish farmers due to the lack of chemical alternatives and herbicide molecules with new action mechanisms. Previously *L. rigidum* Gaud. and *L. multiflorum* Lam. have been confirmed as glyphosate-resistant species in Spain. Since then, glyphosate has been rotated with other herbicides such as oxyfluorfen to improve the control of *Lolium spp*. However, new prospections about glyphosate resistance in *Lolium perenne* L. populations, have also shown oxyfluofen resistance. Such resistance may be influenced by glyphosate resistance acquired previously. The objective of this study was to characterize the resistance shown to oxyfluorfen in *Lolium perenne* glyphosate resistant population. This population was collected in groves that had been treated with oxyfluorfen. Other grasses glyphosate resistant (GR) (*Leptochloa virgate* (L.) Beauv. And *Eleusine indica* (L.) Gaertn.) were compared to susceptible populations (S) of the same species. Glyphosate dose-response assays confirmed the glyphosate resistance in the three species. The species showed high values of reduced growth of 50% (GR_{50}) with resistance factors (RF) between 1.87 and 4.88. The order of resistance was as follows: *L. perenne >L. virgate >E. indica*. Oxyfluorfen dose-response assays showed that the species of *Loliumperenne* was2.1 times more resistant compared to its corresponding S biotype. The GR and S biotypes of *L. virgate* and *E. indica* did not show any level of resistance to oxyfluorfen. These results confirmed a high level of resistance to glyphosate in the three species studied, as well as a new multiple-resistance to oxyfluorfen in *Loliumperenne* population.

However, this resistance was not influenced by the acquired resistance to glyphosate.

Keywords: multiple-resistance, dose-response, perennial crops, glyphosate resistance, oxyfluorfen resistance.

Proactive Management of *Eleusine indica* treated with glyphosate

Fernández-Moreno, P.T.[1]; Alcántara-de la Cruz, R.[1]; De Prado, R.[1]

[1]Department of Agricultural Chemistry and Edaphology, University of Córdoba, 14071 Córdoba, Spain. (pablotomas91@hotmail.es)

Eleusine indica L. is an exotic grass of the Poaceae family, located in temperate climates. This weed produces high reductions in annual crops. It has also been observed in perennial crops recently. As a result of herbicide misuse by farmers, this weed has been described as resistant to different action sites, highlighting cross-resistance to ACCase inhibitors and glutamine synthase inhibitors, among others. In recent years, *E. indica*has been established in citrus groves. Due to the concern expressed by farmers of the province of Castellon (Eastern Spain), who have applied glyphosate in their fields without performing rotations with other active ingredients, dose-response assays and proactive field controls were carried out. Seeds from fields that had been exposed to glyphosate for several years (“E”) were collected and compared with seeds from nearby fields, which were never exposed (“UE”). Dose-response assays showed no significant differences with resistance factors (RF) close to one between both populations. The values of field trials showed significant differences between different herbicides. The order of effective control was the following: Cycloxidim > glufosinate > flazasulfuron > oxyfluorfen > iodosulfuron. These herbicides showed values higher than 85% of effective control. Glyphosate showed a value lower than 80%.These results confirm that at the laboratory level there is still no glyphosate resistance. However, field trials did not produce a good control. This problem can be solved by implementing a proactive management. In order to avoid this resistance which has been reported in other countries.

Keywords: *E. indica*, glyphosate, resistance, proactive management.

Screening maize lines for resistance to nicosulfuron

M.N. Mezili[1], I.S.Livanios[1], P.J. Terzopoulos[2], P.J. Bebeli[1]

[1]Department of Plant Breeding & Biometry, Agricultural University of Athens, Greece (bebeli@aua.gr)
[2]House of Agriculture Spirou SA, Athens, Greece

Sulfonylureas are a group of herbicides which are used to control annual and perennial weeds in maize (*Zea mays* L.). They act by inhibiting AHAS/ALS enzyme (acetolactate synthase or acetohydroxy acid synthase), which catalyzes the first step in the synthesis of branched-chain amino acids. Their movement inside plant tissues is both apoplastic and symplastic, and they have been successfully used for many years, as they combine low application rates and favorable toxicological properties. The aim of this study was to evaluate the sensitivity of nine maize lines (L1-L9) at two different nicosulfuron concentrations (1 and 2 times the recommended dose). Plantlets were subjected to leaf area spraying when the majority of them had reached the fourth leaf stage. Before each treatment, observations of plants' overall appearance were taken to ensure that future measurements would only relate to herbicide effects. Plants were evaluated 7, 14 and 21 days after nicosulfuron application using a 0 to 100 scale, where 0 referred to healthy plants and 100 to completely dried plants. There was a significant effect of nicosulfuron dose on plants' reaction to the herbicide after each measurement, while genotype effect was significant only -one week after herbicide application. Student's t comparison method revealed that lines L3 and L7 showed the highest tolerance to nicosulfuron after spraying application. L4 could also be characterized as tolerant, in contrast to lines L5 and L8, which showed the lowest tolerance across all doses and measurements. There was not significant interaction between nicosulfuron dose and genotype. Herbicide dose seems to be the key factor affecting plant performance. The method described can help in the discrimination among tolerant and non-tolerant genotypes at the early stages of maize cycle.

Keywords: Sulfonylureas, corn, AHAS/ALS enzyme, tolerance, herbicide tolerance.

Metabolism of Imazamox in *Triticum aestivum*

Rojano-Delgado A.M.[1], Jiménez F. [2], De Prado R. [1]

[1] Department of Agricultural Chemistry, C-3 Building, Campus of Rabanales, and Agroalimentary Excellence Campus, ceiA3, University of Córdoba,
E-14071, Córdoba, Spain.
[2] Dominican Research Institute for Agriculture and Forestry (IDIAF)
Rafael Augusto Sánchez, 10147 Dominican Republic.

Imidazolinones have gained a great success in modern agriculture since they were discovered in the 1980's. This is due to their high efficiency in cereal and legume crops, their low mammalian toxicity and low environmental impact, a wide crop selectivity and large number of formulations suitable for pre- and post-emergence. The biochemical resistance/susceptibility to imazamox in two cultivars —resistant (R) named Pantera and susceptible (S) named Gazul— of Clearfield wheat (*Triticum aestivum*) population were studied. Pantera wheat is a soft cultivar from Chile, which is very important because it provides an efficient option to control weeds resistant to herbicides with different action mechanisms. Experiments in a controlled growth chamber showed that the Pantera cultivar presented a high resistance to imazamox with an ED_{50} value of 107.7 g ai ha^{-1} compared to 1.63 g ai ha^{-1} for the Gazul cultivar. The activity of acetolactate synthase (ALS) in leaves extracted from both cultivars showed a higher (12.9 times) resistance to imazamox in Pantera than in Gazul. Metabolism studies showed a higher and faster penetration of imazamox in the Pantera cultivar than in the Gazul one. LC–TOF/MS analysis of imazamox metabolism showed that the imazamox toxic content in leaves and roots is greater in the sensitive cultivar Gazul than in the resistant cultivar Pantera. The Gazul imazamox translocation from leaves to the roots, is also greater and faster, growing proportionally to dose and time. These results suggest that the imazamox metabolism in the Pantera cultivar is a key resistance mechanism that explains such high levels of herbicide tolerated by the plant, together with a second mechanism due to the loss of imazamox affinity for its target site.

Project ERMES: -Development of a reliable rice information system on the basis of remote sensing, in-situ data and crop modeling

D. Katsantonis[1], C. Dramalis[1], A. Kalaitzidis[1], I. Gitas[2], C. Karydas[2], S. Vizantinopoulos[1]*

[1]Agricultural Organization-DEMETER, Cereal Institute, GeorgikisScholis, 57001, Thermi, Thessaloniki, Greece

[2]AristotelioPanepistimioThessalonikis (AUTH), Laboratory of Forest Management and Remote Sensing. School of Forestry and Natural Environment, Administration Building, University Campus, 54124 Thessaloniki, Greece

The main target of the ERMES** project is the development of knowledge and innovation for rice crop aiming at the accurate and timely prediction of rice yield, based on a compilation of data by performing remote sensing technology e.g. satellite radar /optical images, in situ crop data and the analysis of these data by using models. More specifically in-situ information will be provided to the crop model by field operators and/or sensor technology using advance smart applications and technologies. These data will be used to perform crop monitoring, bio-parameters retrieval and meteorological variables estimation. Biotic and abiotic factors and their influence on the crop yield will be also measured. The specific objectives of the project at the European level include: a) the contribution to the Regional authorities for the implementation of agro- environmental policies related to rice crop and the new CAP, b) the support of rice growers on the implementation of sustainable strategies and reduction of the inputs (fertilizers, pesticides, energy) in the field c) early warning to the growers related to dangers due to biotic (pests, weeds, diseases) and abiotic (drought, salinity, high temperatures) stresses and d) the provision to the agri-business of rice crop reliable information related to rice crop, assisting them in the decision making process concerning their commercial and exportation decisions. A further goal of the project is to extend and test the developed system to extra European context in order to verify its capability to predict rice yield in other areas such as Asia & Africa, where rice is a major crop. This might be an important aspect concerning - the EU policy on rice-, since EU may, depending on the annual conditions, adapt its policy (imports/exports) and define goals for its Member States

* Research team for supporting the project in Greece

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Multiple resistance to imazamox and glufosinate and in winter wheat

Rojano-Delgado A.M.[1], Ortega A.[1], De Prado R. [1]

[1] Department of Agricultural Chemistry, C-3 Building, Campus of Rabanales, and Agroalimentary Excellence Campus, ceiA3, University of Córdoba, E-14071, Córdoba, Spain.

The wheat market is of great relevance in the worldwide agricultural economy both because of the cultivated extension and growing number of farms devoted to this crop. Clearfield wheat variety (*Triticum aestivum*) resistant to imazamox has been used in Chile as an effective way to control a large number of weeds tolerant and/or resistant to herbicides. However, the introduction of a resistance to other herbicide in the same cultivar would be a great key point to world the weed control. The aim of this research was to study the resistance mechanisms involved in two cultivars of *Triticum aestivum*. Pantera and Anza were obtained in our laboratory with different degrees of multiple resistance to imazamox and glufosinate. Two parameters (ED_{50} and I_{50}) were used to determine the different responses in the resistance to imazamox and glufosinate of both cultivars compared to the sensitive cultivar (Gazul). The imazamox+glufosinate (1:10) rate necessary to obtain 50% of fresh mass reduction (ED_{50}) was 95.82+ 958.23, 51.73+517.34 and 3.43+34.32 g ai ha^{-1} for Pantera, Anza and Gazul, respectively. For the parameter I_{50} (the herbicide concentration that reduces 50% of the enzyme activity) two enzymes (Acetolactate synthase–ALS– and Glutamine synthetase–GS–) were studied depending on the herbicide used. For ALS enzyme the I_{50} for each wheat cultivar was 74.85, 15.70 and 3.56 µM of imazamox for Pantera, Anza and Gazul cvs, respectively. For GS enzyme the I_{50} was 39.95, 64.98 and 0.62 µM of glufosinate for Pantera, Anza and Gazul cvs., respectively. Although, these new cultivars are resistant to imazamox and glufosinate there are differences between cultivars, which could be explained by different resistance mechanisms.

Selectivity of bentazon and imazamox in faba bean (*Vicia faba*)

C.A. Damalas[1], S.D. Koutroubas[1], T.K. Gitsopoulos[2]and S. Fotiadis[1]

[1]DeptofAgriculturalDevelopment, Democritus University of Thrace, 68200 Orestiada
[2]Hellenic Agricultural Organization-Demeter, Institute of Plant Breeding and Phytogenetic Resources, 57001 Thermi, Thessaloniki
Email: cdamalas@agro.duth.gr

Selectivity of bentazon and imazamox in spring-sown faba bean (cv. Chiaro di Torre Lama) was evaluatedin a field experiment atthe Farm of Democritus University of Thrace in Orestiada.Tworates of bentazon (720 and 1440 g ai/ha) and two rates of imazamox (25 and 50 g ai/ha) were evaluated compared to a non-treated control with weeds (weedy control) and an untreated control without weeds (weed-free control). The selectivity of both herbicides was assessed visually by recording phytotoxicity symptoms on faba bean plants, by measuringfresh weight of plants 7, 14 and 42 days afterherbicideapplication, and by determining grain yield and its components at harvest. The high rates of both herbicides showed some degree of phytotoxicity with appearance of chlorosis and charcoal-grey spots on the foliage (apparent both at the margins and the blades) for bentazon and inhibition of growth (stunting) without other visible symptoms for imazamox. These effects were also confirmed by a reduction in fresh weight of faba bean plants a week after application. The symptoms decreased gradually (14 days) and almost completely disappeared with time (42days). The two herbicides did not affect fresh weight of faba bean plants 42 days after application and further did not affect the number of pods per plant and the number of seeds per plant at harvest. The presence of weeds in the entire growing season (weedy control) reduced grain yield by 39% compared to the weed-free control. Herbicide treatments provided higher grain yields than that of the weedy control, reflecting their efficacy against common lambs quarters.

Field horsetail (*Equisetum arvense L.*) and its effects on chickpea

N. Koustas[1], I.S. Travlos[1], D.J. Bilalis[1], P. Papastylianou[1], D. Papadimitriou, D. Douma[2], D. Chachalis[3] and A. Karkanis[4]

[1] Agricultural University of Athens, Faculty of Crop Science, Athens, Greece
[2] TEI of Epirus, Arta, Greece
[3] Benaki Phytopathological Institute, Kifisia, Greece
[4]University of Thessaly, Department of Agriculture Crop Production and Rural Environment

Field horsetail (*Equisetum arvense* L.) is a member of the Equisetaceae family which was comprised by many species more than 200 million years ago. These plants are considered living fossils, are often promoted for medicinal purpose but sometimes they can be noxious weeds, especially for crops of low height and competitive ability. Field horsetail is a perennial species of high adaptability which propagates mainly by rhizomes. The main objectives of the present study were to evaluate the effects of *E. arvense* on growth and productivity of chickpea (*Cicer arietinum* L.) under field conditions and to investigate some control options of this weed. Therefore, a field experiment was conducted in Domokos region in Central Greece in 2014. Chickpea cv. Thiva was seeded according to the common practices. The experimental area was naturally infested with horsetail. The experiment was arranged in a completely randomized design with three treatments (plots with high and low density of horsetail and weed-free plots) and four replicates. Plot size was 3 x 4 m. Several measurements were taking throughout the growing period. Results showed that horsetail reduced several growth and yield parameters of chickpea, such as root length and number of pods and seeds. It is also noticeable that seed yield was 41 and 29% lower than the weed-free plots, in the case of high and low density of horsetail, respectively. Our results revealed that chickpea can be very susceptible to the competition imposed by horsetail and several measures should be taken for the avoidance of its establishment. These include avoiding of light tillage, improvement of drainage and use of some herbicides like amitrole before the establishment of a new crop.

Screening for resistance to nicosulfuron in maize lines using *in planta* bioassays

M.N. Mezili[1], I.S.Livanios[1], P.J. Terzopoulos[2], P.J. Bebeli[1]

[1]Department of Plant Breeding & Biometry, Agricultural University of Athens, Greece (bebeli@aua.gr)
[2]House of Agriculture Spirou AEBE, Athens, Greece

Sulfonylureas are a group of herbicides which act by inhibiting AHAS/ALS enzyme (acetolactate synthase or acetohydroxy acid synthase), which catalyzes the first step in the synthesis of branched-chain amino acids. They are used to control broad spectrum annual and perennial weeds in maize (*Zea mays* L.). Their movement inside plant tissues is both apoplastic and symplastic, and they have been successfully used for many years, as they combine low application rates and favorable toxicological properties. The aim of this study was to evaluate the sensitivity of five maize lines (L1-L5) at two different nicosulfuron concentrations (1 and 2 times the recommended dose). The herbicide was applied when the majority of plants had reached the fourth leaf stage. Two methods of nicosulfuron application were used: a) leaf area spraying and b) irrigation. Before each treatment, observations of plants' overall appearance were taken to ensure that future measurements would only relate to herbicide effects. Plants were evaluated 7, 14 and 21 days after nicosulfuron application using a 0 to 100 scale, where 0 referred to healthy plants and 100 to completely dried plants. There was a significant effect of herbicide dose across all measurements. Significant interactions of herbicide application x dose were found for the first two weeks and herbicide dose x genotype after three weeks. Combining both herbicide application methods, lines L3 and L4 could be characterized as tolerant to nicosulfuron, in contrast to lines L5 and L1. Germplasm evaluated as tolerant after having been sprayed (L3), demonstrated a similar picture when irrigation with herbicide was applied. Also, the non-tolerant-evaluated genotypes showed similar phenotype behavior. Herbicide dose seems to be the key factor affecting plant performance. The methods described can help in the discrimination among tolerant and non-tolerant genotypes at the early stages of maize cycle.

Keywords: Sulfonylureas, corn, AHAS/ALS enzyme, leaf area spraying, irrigation.