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RESEARCH SOCIETY**

Weed Resistance Working Group Workshop

Abstracts Booklet

***“Perspectives and Challenges of Weed Control and
Weed Resistance to Herbicide in Europe”***

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Early indications of herbicide resistance in UK arable brome grass weeds

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Brome grass weeds are wide-spread across UK cereal growing areas, with indications that incidence and problems with control are increasing. Currently, there are no confirmed UK cases of herbicide resistant brome species, but a number of populations have been identified that are in the process of evolving glyphosate resistance. There are also populations resistant to ALS and ACCase inhibitors in France and Germany, as well as to glyphosate in Australia. Herbicide options for brome control are already limited, so any further erosion of herbicide options through development of resistance is of particular concern. In 2017/18, 63 UK populations of *Anisantha sterilis*, *Anisantha diandra*, *Bromus commutatus*, and *Bromus secalinus* were screened for variation in herbicide sensitivity to two ALS inhibitors, two ACCase inhibitors, and glyphosate. Populations were sent by farmers and agronomists as part of an online nation-wide brome survey. Herbicide sensitivity varied significantly between brome populations and species. Known sensitive populations were completely controlled at recommended herbicide field rates, but a number of populations had high levels of survival when treated with field rates of ALS inhibitors, ACCase inhibitors, or glyphosate. Percent reduction of fresh weight at full field rate of ALS inhibitors ranged from 50-98%, for ACCase inhibitors 41-99%, and for glyphosate 84-97%. Populations suspected of being resistant to ALS inhibitors were tested for target site mutations. One *Bromus secalinus* population was identified with a Pro-197 mutation and one *Bromus commutatus* population was identified with a Trp-574 mutation. Populations were also tested for levels of AmGSTF, a glutathione transferase protein shown to be active in metabolically herbicide resistant black-grass, however results were inconclusive. Glasshouse dose-response experiments for confirmation of resistance to ALS inhibitors, and potentially ACCase inhibitors, are ongoing. If confirmed these will be the first cases of herbicide resistant bromes in the UK and resistant *Bromus commutatus* world-wide.

Monitoring and sampling of herbicide-resistant weed populations

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Herbicide-resistant (HR) weeds in modern agriculture is a serious challenge. How to determine and manage HR weeds is an important question not only for scientist but also for farmers. Monitoring of HR and sampling technique are essential components to define the spread of resistance over local, regional, province and state level. Nowadays, there are two common ways of monitoring: (1) reactive monitoring used for the dissemination purpose of involving HR populations and documenting the incidence, (2) proactive monitoring is to determine resistance before it becomes dominant in a field or widespread across the geographical area. A sampling of HR weeds can be conducted using various methodologies: systematic sampling, random sampling, a combination of both during random sampling in selected locations or sampling in areas where herbicide failure was observed after spraying and this was not associated with application timing, application technique or sub-optimal environmental factors. Knowledge of the history of chemical weed control in commercial fields and the present weed species are important components of the survey. Involvement of local farmers in the survey can assist scientist, agronomist, public and/or private sector to conduct the meaningful widespread geographical survey. The timing for sampling and the number of plants or seeds collected per unit area are the important parameters to decide in addition to what supplemental information needs to be collected during the process. All weed methods practiced at the specific area have a significant impact for monitoring, early detection of HR and then its mitigation. In this review we aim to summarise the appropriate sampling procedures for herbicide resistance assays of grass and broadleaves weeds from suspected resistant and susceptible plants; the general requirements as well as advantages and disadvantages of each procedure.

Keywords: Herbicide-resistant, survey, reactive monitoring, proactive monitoring

Investigations on the determination of glyphosate resistance of horseweed species (*Conyza* spp.) occurring in citrus and vineyards from Mediterranean and Aegean regions

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The main purpose of these studies was to determine the probability of glyphosate resistant horseweed (*Conyza* spp) existence in wine and citrus plantations of Aegean and Mediterranean regions of Turkey, as well as to evaluate the incidence of resistance. With this aim 252 populations were collected from orchards with severe infestation with *Conyza* spp. 42 populations were found suspicious in initial screening tests at 2160 g a.i./ha dose of glyphosate. A dose-response experiment was conducted with seeds collected from fields and another experiment with seeds from suspected plants grown in greenhouse (F2 generation). Results from both experiments showed that six populations from wine and 15 populations from citrus plantations had resistance degrees over two. All these populations were collected from wine and citrus plantations of Mersin province as well as from citrus plantations located in Adana and Hatay provinces of eastern Mediterranean region. These resistance cases were also confirmed by quick test based on shikimic acid accumulation. Although no mutation was detected in molecular studies, there were apparent increases in gene copy numbers of resistant populations. In further studies *Conyza* spp. were molecularly identified and found that 11, nine and one resistant species were *C. canadensis*, *C. bonariensis* and *C. sumatrensis*, respectively. Results of these studies pointed out resistance occurrence and spread risk in the region. However, frequency of resistance was lower than estimated. This shows that besides resistance, control methods applied for *Conyza* spp. in citrus and wine plantations are not suitable to obtain acceptable efficacy.

Key Words: Glyphosate, *Conyza* spp., resistance, citrus, wine, shikimic acid, molecular

Monitoring of the herbicide resistant weeds in Croatia, 2017-2020

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First report of herbicide resistant weeds *Chenopodium album*, *Amaranthus retroflexus* and *Ambrosia artemisiifolia* to atrazine, ciazine and simazine in Croatia dates from early 1980s. To date, weed resistance to herbicides was not often a research subject in our country. However, the Regulation (EC) No 1107/2009 concerning the replacing of PPP on the market led to the withdrawal of the large number of herbicides, which resulted in repeated use of herbicides with the same mode of action. Therefore, Croatian Ministry of Agriculture at the end of last year founded the project “*Monitoring of pest resistance to plant protection products*” with potentially resistant weeds as a part of project. First phase was to contact producers and gather information about suspiciously lacking herbicide efficacy. The online questionnaire was carried out to agricultural producers from each Croatian county (21). The aims were to obtain information about (1) most commonly used herbicides in corn, soybean, cereals and vineyards and (2) suspiciously poorer herbicide control of weed species. Till now, 92 agricultural producers responded the questionnaire and ranged most commonly used herbicides as follow: nicosulfuron in corn (60%), oxasulfuron in soybean (82%), combination of amidosulfuron and iodosulfuron in cereals (51 %) and glyphosate in vineyards (100 %). Suspiciously poorer herbicide control was observed for five weed species: *Ambrosia artemisiifolia* (58%), *Sorghum halepense* (47 %), *Echinochloa crus-galli* (30 %), *Amaranthus retroflexus* (10 %) and *Chenopodium album* (8 %). According to questionnaire results, the second phase was to collect seeds of the above-mentioned suspicious weed species from the producer’s fields. In the upcoming period, herbicide resistance experiments will be conducted in order to test the sensitivity of collected weed species to different rates of most commonly used herbicides (primarily ALS enzyme inhibitors). By the year 2020 complete insight of resistant populations in Croatia should be obtained.

Weed resistance in Lithuania

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Weeds remain the most important factor causing yield reduction. Herbicides seem to be one of major factors determining changes of weed flora. Silky bent grass (*Apera spica-venti* (L.) P. Beauv.) is a common weed of cereal crops widely spread in Northern and Eastern Europe. *A. spica venti* is very frequent in winter cereals in Lithuania as well, especially in the South and Central part. Absence of crop rotation, use of minimal soil tillage and repeated usage of the same active ingredient for weed control were main factors governed decrease of herbicide efficacy and likely resistance. Main herbicides used in Lithuania for *A. spica venti* control belongs to ALS and ACC mode of action.

In previous investigations we found, that lot of populations of *A. spica venti* were resistant to ALS inhibitors: metiljodosulfuronnatrium, pyroxulamandsulfosulfuron. Some populations were resistant not only to one active ingredient, also to two or three, but all populations were sensitive to ACC inhibitors (pinoxaden). The samples of *A. spica – venti* seeds were taken from farmer's fields in 2017 and pot experiment with different rates of pinoxaden was made. Twelve populations of *A. spica venti* were examined. Populationwere chosen after experiment with registered rates of different herbicides. Results of dose response experiment showed, that 3 from twelve populations were resistant to pinoxaden – plants survived in pots with 4X rates of this herbicide. All of those populations were resistant to ALS inhibitors as well.

With growing resistance against ALS and ACC possibility to control *A. spica venti* in spring will strongly be on the decrease.

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Uncontrolled weeds ("escapes") are commonly present in herbicide-treated fields. "Escaped" weeds after treatment can be related to misapplication, e.g., wrong selection of herbicide, low application rate, wrong application timing, and unsuitable climatic condition such as high wind velocity during application and high temperatures after treatment. However, in some cases, particularly in fields where high selection pressure repeatedly imposed on the weed population, control failure may be due to evolution of herbicide-resistant (HR) weeds. Explicit knowledge on the level of resistance and the mechanism(s) involved are obligatory for development of HR-weed management program. Although attempts are made to facilitate the process, the compulsory confirmation of HR is still time consuming and slow process. Testing suspected HR plants require seed collection, overcoming seed dormancy limitations, and treating whole plants pre- or/and post-emergence with the challenging herbicide under suitable climatic conditions, e.g. temperature and light intensity. This might take many months and require testing the plant response to more than one mode of action (MOA) herbicides in order to determine presence of cross- and/or multiple-resistance. Although the information received could be both, qualitative and quantitative, it does not indicate what resistance mechanism(s) is involved. Molecular diagnosis methods being much faster and informative are obligatory for detection of altered target site (TSR) mechanisms. These methods, in spite of their efficient use, request special equipment and skills, which nowadays are available in most laboratories. With the current increase in number of non-target site (NTSR) HR populations reported, special tests are employed to discriminate between NTSR and TSR mechanisms. Current HR diagnosis approaches and methods will be presented, emphasizing their relevance to current HR cases and practical aspects.

Evaluation of the inherent tolerance in *Vulpia myuros* to prosulfocarb at different growth stages

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Vulpia myuros is a recent grass weed problem in some European countries. Due to its inherent susceptibility to soil cultivation, *V. myuros* is most common in no-till cropping systems. *V. myuros* has a natural tolerance to many of the widely used selective herbicides. Previously, mechanisms of tolerance to ACCase and ALS herbicides were reported due to insensitive ACCase and enhanced cytochrome P450 catalyzed metabolism, respectively. Because of the natural tolerance to ACCase and ALS inhibitors, residual herbicides are the only remaining option for chemical control of *V. myuros*. However, low efficacy of residual herbicides like prosulfocarb and pendimethalin against *V. myuros* is often observed in commercial fields. Also, efficacy of residual herbicides tends to decline when applied at later growth stages, therefore, timing of herbicide application is very important.

The objective of this study was to compare the performance of prosulfocarb on *V. myuros* and *Apera-spica ventiat* different growth stages. To this aim, dose response trials were conducted in glasshouse. Various plant growth stages (pre-emergent, germinating seedlings, 1 leaf stage, 2 leaf stage, 3 leaf stage, and 4 leaf stage) were achieved by sowing seeds of both species at different times.

For each plant growth stage dose response curves and ED50 values were estimated. The ED50 values proved that *V. myurosis* more tolerant to prosulfocarb than *A. spica-venti* at all growth stages, and relative difference between ED50 values was constant among two species at early growth stages. Some level of *V. myuros* control was achieved when prosulfocarb was applied at early growth stages. These results highlight the value of optimization of herbicide application timing for maximum control of *V. myuros*. Further investigations are in progress to understand the mechanistic basis of natural herbicide tolerance in *V. myuros*, and to develop integrated management practices for this weed species.

***Amaranthus palmeri* and *Amaranthus tuberculatus*(*rudis*): herbicide-resistant populations
summer weeds**

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Amaranthus palmeri and *A. rudis* are summer annual weeds that due to their vigor, prolific seed production and seed longevity in seed bank cause severe damage to irrigated crops. Numerous reports have shown that these weeds evolved resistance to several herbicide mode of actions (MOA). These alien weed species are dioecious enabling them outcross with other *Amaranthus* spp. ALS-resistant *A. palmeri* was reported more than a decade ago, whereas *A. tuberculatus* has recently started to infest irrigated crops in Israel and there are already ALS-resistant populations across the country. The objectives of this research are: To study the biology and the distribution of *A. tuberculatus* in ruderal and segetal habitats; response to herbicide as compared to *A. palmeri*, and possible outcrossing between the two species. The ALS-resistant *A. palmeri* population was 17 times more resistant to trifloxysulfuron as compared to the susceptible population. DNA sequencing of the ALS gene revealed a target site mutation in position 197. More than 20 populations of *A. tuberculatus* were collected throughout the country and mapped (GIS). Most of the ruderal populations were susceptible to the tested herbicides, whereas those collected in irrigated crops adjacent to *A. palmeri* survived high rates of ALS herbicides, indicating possible outcrossing between the species. Studies on the extent of outcrossing between the two species under different climatic conditions is in progress. The level of *A. tuberculatus* distribution, growth and propagation characteristics found so far imply that special measures should be taken in order to prevent the potential threat of its future infestation in cultivated fields.

Epidemiological approaches in resistance survey and management

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Herbicide resistance has been found to evolve rapidly and consistently in response to herbicide selection in weed populations globally. Historically, resistance surveys have focussed on identifying the presence, extent, and mechanism of resistance amongst weed populations. However, through the integration of contextual management, demographic, and environmental data, we can gain greater insight into the evolutionary drivers and dynamics of herbicide resistance evolution. Using *Alopecurus myosuroides* as an example, we demonstrate the value of such an epidemiological approach for surveying herbicide resistance at a national scale, combining traditional dose-response phenotyping and assays for resistance mechanisms, with population mapping and collection of detailed management histories in over 100 field populations. Using robust statistical methodologies, we evaluate the relationships between current weed population densities, herbicide resistance, and field management histories (herbicide applications, crop rotations and cultivation regimes) to identify the key agronomic factors driving herbicide resistance evolution in this species. Using this framework, we test specific hypotheses about the effect of herbicide use patterns (diversity, mixtures, rotations) on the dynamics of target-site vs. non-target-site mechanisms of resistance. In a separate study, we also used this collection of populations to *pre-emptively* screen for directional selection for reduced glyphosate sensitivity in advance of confirmation of field-evolved resistance. Our work highlights the value of supplementing resistance surveys with epidemiological approaches to infer the key drivers of weed abundance and resistance evolution for traits which have already evolved, and for pro-active assessment of future resistance risks.

Epidemiology and spread of herbicide resistance in rice and HR weed management by new mode of action herbicide, Rinskor

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Evolution of herbicide-resistant (HR) problems reached epidemic levels and lead to driving up crop production costs and decreasing farm profitability. The project aimed to quantified ALS and ACCase resistance occurrence factors and frequencies of *Echinochloa oryzoides* and *Echinochloa crus-galli* in the greenhouse experiments since 2016 growing season. The seeds from 62 fields that survived ALS and ACCase inhibitor exposure collected for 12-yrs and tested in the greenhouse. The field-specific management histories for those fields collected. Rotation practices were a most preclusive factor for ALS and ACCase resistance among the other variables. The other important factor is herbicide mixing which was strongly linked with reduced selection for resistance to that mode of action. Moreover, an important output in studies is the behavior of farmers in managing resistance. Farmers have less inciting to manage resistance because most of them believe another new mode of action herbicide will soon become available to “solve” their problem.

To solve those existing resistance problems, we aimed to develop HRWM programme. Dow AgroSciences is developing Rinskor™ active - a new arylpicolinate herbicide that is synthetic auxin herbicide mode of action and utility in diverse rice cropping systems. This research aimed to create resistance management strategies for the development of sustainable resistance management programs. The experiments were established in fields where rice had been continuously cropped for long periods, and that was infested with *Echinochloa crus-galli*, *E. oryzoides* and *C. difformis* biotypes with confirmed resistance to ALS Inhibitor and ACCase herbicides. Experiments were designed as randomized complete blocks with four replications. Rinskor applied alone, with pre-emergence (PRE) or post-emergence (POST) herbicides combinations with 15 treatments. A combination of PRE application of clomazone and oxadiazon (2 L ha⁻¹) and POST application of Rinskor (2 L ha⁻¹) was the most effective treatment resulting in excellent long-term control of *E. oryzoides* and *E. crus-galli* with significantly fewer weeds before harvest. Also, Rinskor application alone provided excellent control of *Echinochloa crus-galli*, *E. oryzoides* and *C. difformis* when applied twice at 25 g ai/ha with 10-day interval between applications starting at BBCH 11 to 13 of the weeds. Rinskor did not adversely affect rice yield. It is certain that a resistance management program that will be made by relying only on Rinskor will not be sustainable. Therefore, pre-application of a different mode of action like clomazone or oxadiazon has to be included in the resistance management programme. In order to prolong the life of a new mode of action herbicide, economic challenges must be faced by farmers.

Key words: Epidemiology of HR, factors affect occurrence HR, management

Cooperation on herbicide resistance across borders

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There is a need for cooperation across borders to follow the herbicide resistance evolution, to learn from each other and to develop test methods and management strategies. This review lists examples of international collaboration on herbicide resistance.

The International survey of herbicide resistant weeds is a collaborative effort between weed scientists in over 80 countries. The main objective is to maintain scientific accuracy in the reporting of herbicide resistant weeds globally. It is supported by government, academic, and industry weed scientists worldwide

The Herbicide Resistance Action Committee (HRAC) is an international organization operated by members of the agrochemical industry. Their mission is to protect crop yields and quality worldwide by supporting efforts in the fight against herbicide resistant weeds. The work supports regional herbicide-resistance offices around the world.

European Plant Protection Organization (EPPO) is an intergovernmental organization responsible for cooperation in plant health. Its objectives are to protect plants, by developing international strategies against the introduction and spread of pests which are a threat to agriculture. EPPO has produced a large number of 'Standards', among these a standard for resistance risk analysis and is currently developing a database on resistance.

NORBARAG is a Nordic-Baltic collaboration on pesticide resistance research. The network includes representatives of research institutes in Denmark, Estonia, Finland, Latvia, Lithuania, Norway and Sweden and of the agrochemical companies operating in the region. The annual meeting provides a forum for information exchange between people actively involved in research into pesticide resistance and opportunity to discuss strategies to avoid resistance and to manage resistant populations, to define research needs, discuss test methodologies and agree on standards.

Finally, RELIUM is an example of a project promoting collaboration on herbicide resistance across borders. In the project researchers from Italy, Greece and Denmark cooperate at monitoring, mapping resistance cases, elucidating the resistance mechanisms involved as well as developing resistance management strategies for *Lolium* in various agronomic situations.

Management of herbicide resistance: A multidisciplinary problem?

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It is known that farmers are the key to effective herbicide resistance management since they make the final decisions on resistance management strategies used. In reaction to the rapid increase in the number of resistant weed cases, weed scientists and crop consultants have intensified knowledge dissemination concerning herbicide resistance issues, with particular emphasis on the communication of appropriate herbicide resistance management strategies. However, the effectiveness of these educational efforts remains largely non-transparent as the uptake of proposed resistance management strategies is highly influenced by personal experience with resistance and the perception of the effectiveness of a specific management strategy.

Understanding farmers' perception of herbicide resistance issues and factors that drive farmers' adoption of certain resistance management strategies is therefore crucial for the development of sustainable resistance management programs. Sociopolitical research in this area applies at the level of the individual (farmer), the community (farmers in a certain region), and the federal government or nation-state (region/country). Each level requires specific communication strategies and therefore specifically-targeted educational and practical approaches. When it comes to the individual and community level, both the role of distributors and agricultural advisors and the communication between individual farmers become more important. In addition, especially at the individual level of the farmers, decisions are driven by economic hurdles. Calculations of the actual costs related to specific resistance management strategies are still sparse due to their high dependency on farm-specific characteristics and environmental conditions.

Therefore, understanding farmers' behaviors in adopting resistance management strategies requires considerations from a sociopolitical and economic standpoint and therefore calls for a multi- or interdisciplinary approach which will be outlined in the presentation.

Mechanism, distribution and management of herbicide-resistant *Sorghum halepense* biotypes

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Johnsongrass (*Sorghum halepense*), is a troublesome perennial weed infesting summer crops worldwide. Herbicides for selective and effective control of this weed are scarce. This study was initiated following farmers reports on poor control of *S. halepense* with ACCase-inhibiting herbicides, aiming at understanding the magnitude of the problem, the mechanisms involved and examine alternative management practice. Seeds and rhizomes of 17 *S. halepense* populations were collected from fields where chemical control has failed, and grown under controlled conditions. Dose response experiments conducted indicated that two populations are resistant to fluazifop-p-butyl and two populations are resistant to rimsulfuron-methyl. The resistance index of the fluazifop-p-butyl-resistant populations from Yagur and Kfar Blum were 23 and 64 folds more resistant, respectively, than the sensitive population from Ayanot. These populations were equally sensitive to clethodim. A field trial was conducted in Kfar-Blum in a carrot field heavily infested with *S. halepense*, confirmed resistance to fluazifop-p-butyl and other FOP herbicides and sensitivity to clethodim and other DIM herbicides. Molecular analyses revealed that resistant plants possessed an insensitive ACCase enzyme due to a Trp to Cys substitution at codon 2027. Seedling of the rimsulfuron-methyl resistant population from Tel-Nof were 15 folds more resistant than the sensitive population while the resistant population from Hulda didn't vary, mainly due to a low frequency of resistant individuals, suggesting evolution of resistance is still in progress compare to Tel-Nof population. Molecular analyses revealed that resistant plants possess an insensitive ALS enzyme due to a Pro to Thr substitution at codon 197, this is the first reported case in *S. halepense*. Alternative management strategies are also determined, using different mode of action herbicides.

A Case Study Approach in Germany to Study Key Impact Factors and Predict Herbicide Resistance Evolution of Black Grass in Cereal Based Cropping Systems

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Weed control is essential to insure sustainable food, feed, and fibers production. Herbicide resistance is an increasing thread worldwide and is impacting European farmers; especially those relying on cereal based cropping systems. However, little is known about the exchange of resistance genes between neighboring fields and the underlying field management and ecological factors. Therefore a project was initiated in Germany in 2010 to study temporal and spatial evolution of resistance to ALS- and ACCase-inhibitors in black grass (*Alopecurus myosuroides* Huds.). The study includes 120 fields for which seed samples were collected over a 6 year period.

Assessment of field survivors in the greenhouse revealed that over 83% of the samples showed resistance to ACCase-Inhibitors of the FOP/DEN-classes. Less than 32% showed resistance to mesosulfuron-methyl (ALS-Inhibitor). The degree to which resistance was pronounced and the pattern of the SNPs analyzed (2 ALS; 5 ACCase) varied from field to field and from year to year, suggesting that resistance develops locally on the individual field level. Surprisingly, the number of Modes of Action used seemed to have a weak significant effect on the resistance evolution to ALS inhibitors after 2-3 complete crop rotations. In addition, the effect of the herbicide use frequency and the use frequency of ALS- inhibitors was also less pronounced compared to the non-chemical weed management measures. However, similar management practices were not necessarily leading to the same resistance status within the small area assessed in this study. Deeper analyses showed that also particular soil types are correlated with the presence of ALS-resistant black grass populations.

An attempt to generate a machine learning risk prediction tool based on field history in combination with a population simulation tool was started. Its prediction quality and application for practical agriculture will be discussed in the light of Integrated Weed Management.

Harvest weed seed control- popularity, types, problems and usefulness in Europe

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Harvest weed seed control has been enthusiastically implemented on the majority of cropping farms throughout Australia over the last 10 years as part of their IWM, however there is no targeted HWSC use in Europe. I will examine why HWSC became so popular in Australia, and the role HWSC can play in future IWM in Europe.

Farmers using harvest weed seed control in Australia can now choose from a range of six alternate methods of HWSC, which are suited to different farming practices. I will outline two of these practices, which would be most practical for European farmers, including their theory, advantages, and disadvantages.

Finally I will discuss the selection pressures and evolutionary changes which may result from using HWSC. This section will focus on the indirect effects of HWSC on herbicide resistance evolution, and the more direct effect of HWSC on selection pressure to avoid (become resistant to) seed capture at harvest.

Status of herbicide resistance in annual weedy grasses in the Czech Republic – results of long-term monitoring and management

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Annual weedy grasses are recently the most troublesome weeds of winter cereals in the Czech Republic. Our Department has been continuously monitoring and mapping the resistance with focus on weedy grasses. The samples for testing are collected in the framework of research projects or delivered by different stakeholders from fields with suspected resistance. Simple growth assays in pots with 1N and 2N of registered field rate, or dose response assays are the main methods used for identification of herbicide resistance for this purpose. The results are presented in specialised maps and shared with the national state phytosanitary administration, which present the data in maps on their Phytosanitary portal together with cases of resistance to other pesticides.

The economically most important resistant weedy grass, *Apera spica-venti*, has been monitored for almost 15 years when the first case of resistance against the chlorsulfuron with cross resistance to other sulfonylureas was found. Very soon, in 2005, the multiple resistance to ALS inhibitors (a.i. chlorsulfuron, iodosulfuron, mesosulfuron) and PSII inhibitor (isoproturon) developed, and also the biotype with multiple resistance to ALS and ACCase inhibitors was found. In 2016, we identified and recently are working on the molecular basis of triple resistance to pyroxsulam (ALS), chlorotoluron (PS II) and pinoxaden (ACCase inhibitors) which is the newest phenomenon in agricultural practice.

Creeping problem is the ALS resistance in *Bromus sterilis*. This resistance is not widely spread across the country up to now, but it affects almost all fields in single farms in which this kind of resistance has been identified. The affected active ingredients are both pyroxsulam and propoxycarbazone-Na, the most frequently used herbicides. Because there are no other modes of action available against the *Bromus*, it is expected an increase of resistant populations in the future and difficulties in its control.

Very difficult grass, fortunately with occurrence only in few specific landscapes, is the *Alopecurus myosuroides*. Regardless of limited occurrence, ALS and ACCase resistance is very common in its populations and farmers have to use more expensive herbicide solutions combining autumn and spring control.

The best situation is still among *Avena fatua* populations. Only few cases of resistance have been found to ACCase or rarely ALS inhibitors. Rotation of modes of actions (ALS inhibitors in winter cereals and ACCase inhibitors in spring cereals) probably delays the resistance. Lower share of spring crops in crop rotations is a factor limiting an increase of population densities of this species.

Other grass species, such as *Poa annua*, *Lolium* spp., and *Vulpia myuros* cause economic problems only locally and problems with resistance have not been reported yet.

Because of loss of many products (trifluralin, isoproturon), limited availability of products with pendimethalin, expected limitations for flufenacet, and a political pressure to ban or substantially restrict the glyphosate use, the resistance management in weedy grasses becomes more difficult.

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Herbicide Resistant Weeds in the Çukurova Region of Turkey

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Çukurova Region is one of the main agricultural area in Turkey and has an intensive agrochemicals consumption. Among these chemicals, the herbicides are one of the most used pesticide group. The first observation on herbicide resistance was started in 1993 with wild oat (*Avena sterilis* L.) in the region. After confirmation of resistance on wild oat, wild mustard (*Sinapis arvensis* L.) was found resistant against ALS herbicides. The first multiple resistance from the region was confirmed on Shortspike canary grass (*Phalaris brachystachys* Link.). This resistance was the first time recorded both in Turkey and the world. In the same year, 2008, *A. sterilis*, fox tail (*Alopecurus myosuroides*) and *S. arvensis* were occurred multiple resistance.

After 2010, we decided to create herbicide resistance map starting from the wheat fields of Adana province collaboration with the ministry of agriculture and pesticide companies. In 2011 and 2012, 679 wheat fields were examined and sampled according to the method. The first ALS (Acetolactate synthase) ve ACCase (Acetyl-CoA Carboxylase) resistant *A. sterilis* and *S. arvensis* maps were created in the wheat fields of Adana province. Similar maps were completed for *A. sterilis* in the wheat fields of the Osmaniye province in 2017. The conformation tests which we use were quick tests (agar and paper/sand test), molecular assay in laboratory and whole plant assay in greenhouse. Meanwhile, there are some problems and complaints on *Conyza* spp., *Amaranthus palmeri* and *Euphorbia* spp. from producers. Future researches will be conducted on these weeds.

First case of glyphosate-resistant junglerice [*Echinochloa colona* (L.) Link] in Greece

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The present study was conducted due to several incidents of failure of glyphosate on *Echinochloa colona* in olive and citrus orchards of western Greece. The main objectives of this research were to

determine the potential occurrence of glyphosate-resistant populations of junglerice from Greece, as well as, to evaluate the effect of the weed growth stage on glyphosate efficacy. Fourteen Greek populations of junglerice, sampled from three regions, were studied under controlled conditions. After the initial screening, specific populations were selected and dose–response experiments were conducted with increasing doses of glyphosate (0.045, 0.09, 0.18, 0.36, 0.72, 1.44, 2.88, and 5.76 kg ae ha⁻¹). Glyphosate resistance was confirmed for the first time in Greece in both plant survival and biomass response with the ET6 population being about 4-fold more resistant than the susceptible population KO2. Moreover, our results revealed a different susceptibility of the glyphosate-resistant populations at different growth stages and therefore it is suggested as an important factor to be taken into account.

How glyphosate resistance is modified by exogenous salicylic acid's application on *Conyza bonariensis*

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Glyphosate is the most important herbicide worldwide, and *Conyza* sp. is one of the most common weed species that has developed resistance to it in various parts of the world, including Greece. It is an absolute necessity to investigate the mechanisms of resistance that weeds have developed in order to reduce that phenomenon. Although the effect of salicylic acid for resistance in biotic factors has been studied extensively, the data about its role about resistance to abiotic stress and especially for herbicides are few.

In the present study, an effort has been made so as to study the salicylic acid's impact to glyphosate on *Conyza* sp. weed plants. Results are presented regarding the application of salicylic acid to sensitive and resistant populations of *Conyza bonariensis* at the same time as glyphosate spaying. Measurements were taken as specific indexes as follows:

1. proline concentration
2. hydrogen peroxide concentration (the most important of the active oxygen species - Reactive Oxygen Species)
3. malondialdehyde (MDA) (one of the most reliable indicators for the degree of lipid peroxidation of cell membranes)
4. shikimic acid

The above measurements took place on the 1st, 6th and 10th days after glyphosate application, except shikimic acid, which concentration was estimated three days after herbicide's application. The results showed that sensitive populations with simultaneous application of salicylic acid and herbicide, had an increased level of resistance indexes to glyphosate, since there was a significant reduction at the concentrations of peroxide and MDA as well as an increase at the concentration of proline, in contrast to the sensitive populations, where only glyphosate was applied. The results indicate that salicylic acid could play an important role in the mechanism of glyphosate resistance and deserves further study of the phenomenon.

Resistance of *Geranium molle* to herbicides in orchards

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Geranium molle is an annual herbaceous plant 5 to 30 cm tall. It is a native of the Mediterranean and sub-Mediterranean areas but has naturalized in other parts in Europe, in southwestern and central Asia and in North America. In Israel, flowering from February to May, most commonly in ornamentals, and various fruit tree orchards. In orchards, non-residual and residual herbicides are regularly applied before the winter rains. These herbicide regime contributes to a strong selection pressure on the weed populations, resulting in evolution of herbicide-resistant (HR) weeds. Because of the overuse of glyphosate and increased number of HR weeds in the world, the three MOA herbicide formulated as "glider" was offered to orchards growers in Israel. Recently, we identified populations of *Geranium molle* that under field conditions demonstrate reduced response to glider. This formulation containing oxyfluorfen, glyphosate and diuron was applied repeatedly for several years in orchards in north Israel. Greenhouse and laboratory experiments have shown reduced control of *G. molle* to diuron (PSII) in one of the collected populations. We assume that since diuron is the herbicide with the longest residual component in 'glider', it will be the first to evolve resistance. In addition, fast recovery was detected in glyphosate treated plants following glyphosate application in the same population. However, no recovery was detected in oxyfluorfen treated plants. These data indicate the risk associated with mixtures of herbicides having different MOA but also different residual activity.

Determination of the Glyphosate Resistant Horseweed Biotypes in Apple Orchard in Isparta, Turkey

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Isparta is one of the prominent city in terms of apple productions in Turkey. Glyphosate has been used to control weeds in apple orchards for many years, and there have been taken many complaints by growers because of its inefficacy. This study was conducted to determine the Glyphosate Resistant Horseweed (GRH) biotypes in Isparta, Turkey. A sixty-eight-horseweed population was collected from the natural fields and apple orchards and screen test was employed to determine GRHs. The growth chamber experiment was conducted at 18 - 21 ° C (8 h light - 16 h dark) conditions. The individuals belonged two GRH population survived after screen test and a Glyphosate Sensitive Horseweed (GSH) were tested using dose-response test. A logarithmic dose series from 1/4X to 16X, X: recommended rate of glyphosate: 1.44 kg ai ha⁻¹, was applied using by spray chamber to horseweed seedlings at 2-4 true-leaves stage. Glyphosate applied seedlings were cut above the ground 28 days after treatment and weighted after dried by analytical digital scale. The data was evaluated using non-linear regression analysis in R statistical programme. The I₉₀ values of GRH and GSH were 1.82 ve 2.34 kg ai ha⁻¹ and 0.557 kg ai ha⁻¹, respectively. Management of GRH in Isparta, Turkey may not be problem in the short run because of using higher glyphosate application rate, 2.34 kg ai ha⁻¹, to control perennial weeds, initiating to use soil residual herbicides and putting into practice mechanical weed control practices effectively.

Key words:Horseweed, glyphosate, herbicide resistance, bioassay

Herbicide resistance in wild oats worldwide and future in Europe

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Wild oats (*Avena* spp.) are among the worst weeds worldwide and distributed through temperate, arid and sub-tropical regions in mainly cereals and many other crops as well as non-cropped areas. Two species of wild oats are common weeds in Europe: *A. fatua* and *A. sterilis* which has two subspecies: *substerilis* and *subpludoviciana*. So far more than 74 herbicide resistance cases in wild oats have been reported since the first case verified in wheat fields in Australia in 1985. In addition to wheat fields, resistant populations have been recorded in croplands such as canola, chickpea, clover, faba beans, lentils, lupins, potatoes, peas, barley, sugar beets where wild oats had been a common weed. Resistance to one or more ACCase herbicides is the most common cases followed by ALS resistance, which multiple resistance with both groups has tended to increase. In addition, resistance to flupropr (an antimicrotubule mitotic disrupter) has been detected at ACCase resistant biotypes in Australia, Canada (including ALS resistance), Turkey and UK. In Canada, triallate (a lipid inhibitor) resistance also reported. In addition, populations resistant to triallate in Canada have multiple resistance up to five sites of action: with cell elongation inhibitor (difenzoquat biotype) resistance (in the USA also), with ACCase and ALS resistance, with ACCase, ALS, and antimicrotubule mitotic disrupter resistance, and with ACCase, ALS, PPO and long chain fatty acid inhibitor resistance. The first case in Europe was reported in 1992, which was ACCase resistant *A. sterilis*. Resistant wild oat has been recorded from Cyprus, Belgium, France, Germany, Italy, Turkey, and UK. In Europe and the Mediterranean Basin there might more cases than it has been reported. The area with herbicide resistant wild oats has not been precisely quantified. Range expansion of wild oats and increasing threat with non-target site resistance cases due to climate change will create bigger problem in Europe. Lack of new herbicides and tools requires innovative integrated management approach to deal with these biotypes.

Glyphosate resistant weeds, impact on grapevines and the occurrence in Northwestern Marmara Region

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Weed control is an important practice in vineyard management. Chemical control with herbicides is most preferred method which can affect weeds under vine, save time and labour. The majority of grapevine producers use herbicides containing glyphosate against broad and narrow leaved weeds. After application glyphosate is absorbed onto soil particles and remain as a residue for several years. In addition this compound is toxic to grapevine and cause abnormal plant growth. Intensive use of this chemical lead to emergence of resistant weeds which can survive and reproduce after lethal dose of herbicide application. Until now several weed species have been reported to have glyphosate resistance in the world. The weed flora of Northwestern Marmara include several of these species which are *Conyza canadensis* L., *Ambrosia artemisiifolia* L., *Plantago major* L., *Sorghum halepense* L., *Galium aparine* L., *Lactuca serriola* L. Within these species *Sorghum halepense* was most common with 63% distribution.

Keyword: glyphosate resistant weeds, Northwestern Marmara Region

Impact of Crop Rotation on Development of Herbicide Resistance in Crop Plants

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Herbicide resistance is the ability of a plant to survive and reproduce following exposure to a lethal dose of herbicide. When the same herbicide is applied every year some weeds develop genetic resistance to herbicide groups. The lethal dose of application will kill susceptible plants but resistance gained survive and reproduce. Herbicide resistance very distributed in some weed genera including *Amaranthus* spp., *Chenopodium* spp., *Conyza* spp., *Lolium* spp., *Setaria* spp., *Echinochloa* spp., *Alopecurus* spp., *Senecio* spp., *Polygonum* spp. and *Solanum* spp. all around the world. Crop rotation and application of different herbicides can retard resistance formation to herbicides. Weed species have different emergence periods. Some species emerges most readily in early spring and is often a problem in summer crop plants. In contrast species like *Lamium amplexicaulis* germinate in fall and become problematic in winter crops. Each herbicide is effective on specific weed species. Crop rotation will alter the diversity of weed species. This method may suppress several weed population. Crop rotation creates opportunities for herbicide rotation as well. To control weeds herbicides with different mode of action will be used. As a result of these actions herbicide resistance development in weeds can be slowed down. But it must be taken into consideration to select rotation crops based on effective herbicides. Rotation within a group of herbicide-resistant crops may lead to an increased risk of selecting herbicide resistant weeds.

Keyword: Herbicide resistance, crop rotation, impact

Status of weed resistance in Spain

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Weed resistance cases in Spain are increasing year after year. The Comitee for Preventing Herbicide Resistance (CPRH) is a working group integrated in the Spanish Weed Research society (www.semh.net) and intends to have an updated list of all resistance cases confirmed around the country.

Confirmed resistance cases actually are:

-*Alisma spp.*, *Cyperus difformis* y *Echinochloa spp.* to group B herbicides in rice

-*Alopecurus myosuroides* to group A herbicides in cereal crops in Navarra and Catalonia regions.

-*Avena sterilis sp. ludoviciana* to group A & B herbicides in cereal crops in Navarra, Rioja regions and the province of Burgos.

-*Chenopodium Album* to triazines in maize and sugarbeet in Castilla y Leon region.

-*Conyza spp.* resistant to glyphosate in perennial crops around all the country.

-*Lolium rigidum* to A, B and C2 herbicides, including multiple resistant biotypes, in cereal & OSR fields around all the country.

-*Lolium rigidum* resistant to groups A, B and & in perennial crops.

-*Papaver rhoeas* resistant to groups O & B in cerea crops in the center and north of the country.

-*Sinapis Arvensis* and *Rapistrum rugosum* resistant to group B herbicides in the north of Navarra and Aragón regions

-*Sorghum halepense* resistant to group B herbicides in maize crop in Aragon, Catalonia, Madrid and Castilla-La Mancha regions

In addition, there are several maize fields where problems with group B herbicides were reported against *Echinochloa crus-galli*.

Taking into account the acreage affected, the main problem is *Lolium rigidum* in arable crops and *Conyza spp.* in perennial crops.

Screening of sterile oat (*Avena sterilis*L.) populations for resistance to ALS inhibiting herbicides, in Greece

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Sterile oat (*Avena sterilis*) is one of the most widely distributed and harmful weeds in winter cereals grown in Greece, routinely controlled with ACCase and ALS inhibitors. However, in 2016 mesosulfuron+iodosulfuron failed to adequately control this species in winter wheat (Serres, Northern Greece) prompting the current study. Seeds from four *A. sterilis* populations (R1, R2, R3, R4) with possible resistance and one from the farm of Aristotle University of Thessaloniki (S, susceptible) were collected. These were evaluated in a pot experiment for possible resistance to ALS inhibitors at 0, N and 4N (N= recommended field dose: mesosulfuron+iodosulfuron 7.5+7.5 g/ha + 0.2% v/v adjuvant, pyroxsulam 15 g/ha + 0.2% v/v adjuvant) with four replicates (five plants/replicate). Pinoxaden applied at N (45 g/ha) was included as a non-ALS chemical control. Herbicides were applied at the four-five leaf growth stage. Six weeks after treatment, plants were visually scored (VS) on a ten-level scale (0=dead, 10=as untreated control) and cut at soil level for fresh weight (FW) measurement. Leaf samples from surviving plants with the highest VS at 4N were collected for ALS genotyping. Mesosulfuron+iodosulfuron reduced the fresh weight of two *A. sterilis* populations (R1, R3) by 9-15% and 24-28% at N and 4N, respectively. Pyroxsulam reduced their fresh weight by 27-32% at N and 35-39% at 4N. Furthermore, pinoxaden reduced their fresh weight by 53-58%. In the remaining two *A. sterilis* populations (R2, R4) mesosulfuron+iodosulfuron at N reduced the fresh weight by 67-70%, while pyroxsulam at the same dose by 67-69%. The efficacy of pinoxaden against these populations was 79-92%. All treatments provided 100% control of the susceptible (S) population. The VS of populations R1 and R3 treated with mesosulfuron+iodosulfuron or pyroxsulam at N, were similar to the untreated control. Further work is underway, with dose-response experiments, ALS sequencing and enzyme activity assays.

Keywords: *Avena sterilis* L., ALS inhibitors, herbicide resistance

**First case of multiple resistance of Johnsongrass (*Sorghum halepense* (L.) Pers.)
to ALS- and ACCase-inhibiting herbicides in Serbia**

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Abstract

Johnsongrass (*Sorghum halepense* (L.) Pers.) is one of the most troublesome weeds in Serbian field crops production. Cross resistance of *S. halepense* in Serbia to some ALS-inhibiting herbicides has been confirmed in 2014., and to some ACCase-inhibiting herbicides has been confirmed in 2017. The aim of our study was to confirm the existence of multiple Johnsongrass resistance to the ALS- and ACCase-inhibiting herbicides.

Greenhouse trials were conducted in 2017 and 2018 to determine the resistance in the suspected Johnsongrass populations from Bač municipality (western Vojvodina province, north Serbia) to the selected ACCase- and ALS-inhibiting herbicides. To determine the distribution of resistance, seeds of 20 Johnsongrass populations were collected from fields where poor efficacy of ACCase-inhibiting herbicides had been reported. Seedlings from those populations were subjected to post-emergence use of two rates of fluazifop-p-butyl (1x=120 and 3x=360 g ai ha⁻¹) and nicosulfuron (1x=30 and 3x=90g ai ha⁻¹). Herbicides were applied at two-three leaves growth stage of *S. halepense* seedlings, with portable sprayer. Shoot fresh weight and % of surviving plants per pot and visual growth reduction in relation to the untreated control were recorded 14 days after the herbicides' applications, respectively. A population is considered as resistant (R) to a herbicide when more than 20% of treated plants survived the recommended herbicide field rate (1x) and highly resistant (RR) when survivors were more than 20% at dose 1x and more than 10% at dose 3x.

Continuous use of some ACCase and ALSase inhibitors in long monoculture of soybean in Bač municipality has resulted in the selection of cross resistance in Johnsongrass to aryloxyphenoxypropionates (FOPs) herbicides and multiple resistance to ALS inhibitors and FOPs. Of the total of 20 populations, resistance or highly resistance to fluazifop-p-butyl was determined in 13 populations concentrated on a territory of about 10 km in diameter. Dose-response bioassays confirmed that the level of resistance as a ratio of R/S based on ED₅₀ (50% fresh biomass reduction) varied between 3.1 (for clethodim and cycloxydim) and 91.2 (for fenoxaprop-P-ethyl). Regardless of the increased RI, the recommended field doses of cyclohexandiones (clethodim and cycloxydim) are effective in controlling resistant Johnsongrass populations on FOPs herbicides. Results from whole plant bioassays confirmed that two of 13 populations resistant to FOPs herbicides, are also resistant to nicosulfuron. One population (SORHA 001-17) was highly resistant (RR) with 50.5% and 10.8% of survived plants after use of 1x and 3x rates of fluazifop and nicosulfuron.