

## MEETING

### ABSTRACTS OF PAPERS PRESENTED AT THE 15TH CONFERENCE OF THE WEED SCIENCE SOCIETY OF ISRAEL

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#### A: BIOLOGY OF PARASITIC WEEDS – BROOMRAPE AND DODDER

##### **Effect of Carrot Sowing Date on Parasitism of *Orobanche crenata* and *O. aegyptiaca***

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The growing season of carrot is all year long, whereas *Orobanche crenata* and *Orobanche aegyptiaca* parasitize this crop only in specific seasons. The aim of this study was to examine the effect of sowing dates on the parasitism level of those two *Orobanche* species. Experimental plots were sterilized with methyl bromide and then artificially infested with the mentioned *Orobanche* seeds; non-infested plots were used as a control. Carrots were sown at the beginning of each month from September 1995 to March 1997. Those sown at the winter sowing dates were parasitized by the two *Orobanche* species, which caused severe damage reaching 50% yield reduction in the cold winter months. However, carrots sown at the spring–summer sowing dates were free of *Orobanche* parasitism. These results exhibit a requirement for low temperatures for *O. crenata* and *O. aegyptiaca* parasitism on carrot, in spite of parasitism of the latter on other summer crops. Presumably, carrot resistance to *O. crenata* and *O. aegyptiaca* is expressed only in the summer growing season. Furthermore, the parasitism process is significantly slower at the autumn sowing dates than in the winter because of the requirement of cold temperatures for parasitism. In laboratory studies, *Orobanche* seeds exhibited a high germination rate when preconditioned at high temperatures and germinated by GR<sub>24</sub>, proving that this resistance is non-dependent on parasite germination ability. In spite of the fact that carrot yields in summer are lower than in winter, carrot growers could evade *Orobanche* damage by advancing or delaying the carrot growth season. In conclusion, it was demonstrated clearly that carrot has seasonal resistance to *O. aegyptiaca* and *O. crenata* which is expressed at summer cropping and disappears at low temperatures. The precise conditions for resistance of carrot to *O. aegyptiaca* and *O. crenata* are presently under study in phytotron experiments.

##### **Effect of Temperature on Host–Parasite Relationship in *Orobanche* spp.**

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In previous host-range experiments we have shown that tomato (*Lycopersicon esculentum* L.) exhibits differential seasonal susceptibility to *Orobanche crenata*. However, eggplant (*Solanum*

*melogena* L.) is parasitized by *O. aegyptiaca* all year round but is not susceptible to *O. crenata*. The aim of this study was to examine the effect of temperature on susceptibility of eggplant and tomato to *O. crenata* and *O. aegyptiaca*.

In field experiments we studied the effect of planting dates on parasitism by *O. crenata* and *O. aegyptiaca* on tomato and eggplant. The field was fumigated with methyl bromide and artificially infested with *O. crenata* and *O. aegyptiaca* seeds, excluding the non-infested control plots. Eggplant and tomato were planted at the beginning of each month from August 1995 until December 1996. Significant parasitism of *O. crenata* was observed on winter-grown tomato which caused a 50% yield loss. Summer-grown tomatoes were not parasitized by *O. crenata*, whereas eggplant was not parasitized by it at all. *O. aegyptiaca* parasitized both crops in both seasons and caused severe damage, reaching total yield loss; high temperatures accelerated the parasitism. In pot experiments conducted in a phytotron we obtained quantitative data regarding the effects of temperatures and thermoperiod (simulating seasonal temperatures) similar to those obtained in the field experiments. In laboratory experiments eggplants and tomato were planted in the polyethylene bag system. Tomato and eggplant induced germination of *O. aegyptiaca* and *O. crenata* seeds at all tested thermoperiods. Higher temperatures increased seed germination of both *Orobanch*e species on both host roots; however, only *O. aegyptiaca* parasitized both hosts' roots.

In conclusion, our study clearly indicates susceptibility of eggplant and tomato to *O. aegyptiaca* that increases at high temperature. Eggplant was resistant to *O. crenata* under all test conditions in spite of its ability to induce seed germination. However, tomato exhibited susceptibility to *O. aegyptiaca* at all seasons or examined thermoperiods, especially at high temperatures. In contrast, *O. crenata* did not parasitize tomato at high temperatures. In this case the tomato roots exuded the germination stimulant inducing *Orobanch*e seed germination, but the parasite's radicals failed to attach to the host root.

### **Diagnosis of Soilborne *Orobanch*e Seeds**

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*Orobanch*e species differ in their host preference. Precise identification of *Orobanch*e is therefore necessary on the species level in order to avoid planting host plants in *Orobanch*e-infested fields. Species determination of *Orobanch*e seeds was hitherto based on their morphology. This method is not satisfactory for soilborne seeds because the surface characteristics are lost in soil by the activity of microorganisms. The use of DNA fingerprinting overcomes this difficulty and enables precise determination of the species of each tiny seed of the parasite. We extracted DNA out of single soilborne seeds and amplified specific DNA regions by use of PCR. At first we selected primers that distinguished the different species. Then we identified PCR products that were specific to single species and verified their specificity by Southern hybridization. We determined the sequence of each of these products, and selected respective SCAR primers with the help of a computer program. The SCAR method allows the precise diagnosis of single seeds. The specific primers for *Orobanch*e *crenata* give a single band at 1400 bp, the primers for *O. aegyptiaca* – at 470 bp, and those for *O. cumana* – at 660 bp. DNA of satisfactory quality can be extracted from viable (tetrazolium-positive) soilborne seeds, as well as from non-viable (tetrazolium-negative) seeds, as long as they contain an intact embryo. This molecular method of *Orobanch*e seed diagnosis enables reliable seed identification on the species level in a short time and at reasonable price.

### **Studies of the Resistance of *Vicia atropurpurea* to *Orobanch*e *aegyptiaca***

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*Orobanche aegyptiaca* and *O. crenata* heavily parasitize vetches in Israel and other sites in the Mediterranean region, causing severe yield losses to these crops. In earlier studies we have shown that common vetch (*Vicia sativa*) is susceptible to *Orobanche*, whereas purple vetch (*V. atropurpurea*) is resistant. The aim of the present study was to investigate the basis for the differential sensitivity of vetch genotypes to *O. aegyptiaca*. Resistant purple vetch (cv. 'Popany') and susceptible common vetch (cv. 'Yovel') were grown in a polyethylene bag system, infested and non-infested with *O. aegyptiaca* seeds. Root sections were excised at three dates and taken for biochemical analyses and anatomical observations. Free and bound phenolics, lignin and peroxidase were highly induced in the *O. aegyptiaca*-infested resistant vetch as compared with the susceptible genotype 'Yovel'. The enzyme phenylalanine ammonia-lyase was not induced, but higher constitutive levels were detected in the resistant than in the susceptible vetch roots. Anatomical studies of the parasite's radicle penetrating vetch roots showed that in the resistant vetch, the radicle is repelled from the vascular cylinder, thus ceasing further parasitism. These findings suggest the involvement of secondary metabolism in the plant defense mechanism. Accumulation of phenolics and lignification of cell walls in the resistant vetch roots, form mechanical and chemical barriers, whereas the phenolic precursors of lignin may prevent the intrusion of the parasite's radicle and further host-parasite association. The isolation and identification of a proposed phytotoxin secreted by the resistant vetch root cells is currently under investigation.

### **Proteolytic Activity of Germinating *Orobanche aegyptiaca* Seeds Controls the Degrading Level of Its Own Excreted Pectinase and Cellulase**

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*Orobanche* (Orobanchaceae) is a nonphotosynthesizing root holoparasitic angiosperm. Its spreading during recent decades, in the Mediterranean area, constitutes an economic threat, unsolved by agronomic technology and herbicide use. Understanding the involved parasitic mechanisms is a must. Germinating seeds of *Orobanche aegyptiaca* were found to excrete cell-wall-degrading enzymes that ensured smooth penetration with minimum damage to the host root. The enzymes were unstable and had very low activity. We searched for a regulating mechanism. Working under sterile conditions, germinated *Orobanche* seeds were exposed to BSA and assayed by the Lowry method, for protein level at time zero and after 24 h. Proteolytic activity was detected that was restrained in the presence of a protease inhibitor. Exposing germinating *Orobanche* seeds to the presence of polygalacturonic acid or carboxymethyl-cellulose, with or without the protease inhibitor, revealed an increase in pectinase and cellulase activity in the presence of the inhibitor. Thus, the results point towards the proteolytic activity as the main regulating mechanism for minimizing host root damage and against self-destruction.

### **Involvement of Pectinases in Plant Infection by Parasitic Weeds**

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*Orobanche aegyptiaca* and *Cuscuta campestris* plants were grown *in vitro* and shown to have the ability to infest host plants. These cultures served as a source for pectolytic enzymes, the most

important of which are pectin methylesterase and polygalacturonase. We assumed that these enzymes weaken and degrade the pectin in the middle lamellae of the host and thus enable the cells of the parasitic plant to penetrate host tissue without damaging it. We showed that *Orobanch*e seedlings secrete both enzymes. Using specific antibodies against these enzymes we demonstrated under the electron microscope pectin degradation in host cell walls in infected tissues. The two enzymes from *Orobanch*e were characterized and purified. The presence of inhibitors of polygalacturonase was demonstrated in *Orobanch*e tissues, and we propose that they are involved in the regulation of enzyme activity during haustorial penetration. In *Cuscuta* several pectin methylesterases were demonstrated. One of them, which is active at pH 7.0, was partially characterized. It differs from most pectin methylesterases described previously.

*B: CONTROL OF PARASITIC WEEDS – BROOMRAPE AND DODDER*

### **Control of *Orobanch*e in Tomatoes with Sulfonylurea Herbicides**

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Germinated *Orobanch*e *aegyptiaca* is controlled by sulfonylurea herbicides in the soil. The herbicides were active on parasitic young stages already attached to the host roots as well. We found that it is possible to inject low rates of chlorsulfuron (Glean, 75% WG) or triasulfuron (Amber, 75% WG) into the tomato root-zone by chemigation *via* sprinkler irrigation and thereby achieve selective control of the parasite. The host/parasite relationship was monitored in the greenhouse and as a result we were able to achieve effective and selective *O. aegyptiaca* control in tomato fields by a split application of three portions of 2.5 g/ha each of chlorsulfuron or 7.5 g/ha each of triasulfuron, applied on established plants 2–3 weeks after planting and subsequently at 2-week intervals. Applying the herbicides at the same rates and timing by directed sprays, or on the foliage in high spray volume (900 l/ha), followed by sprinkler irrigation to wash the tomato foliage, was less effective in *Orobanch*e control and damaged the crop. After changing the irrigation system from sprinkler to dripper, some broomrapes emerged around the drippers as a possible result of pushing or breaking down the herbicide. We succeeded in preventing this phenomenon by additional injection of chlorsulfuron *via* the drip irrigation system, at 0.75 g per 1000 drippers. Applying chlorsulfuron or triasulfuron preplanting to tomatoes was not selective enough for tomato, but triasulfuron was the relatively safer.

### **Selective *Orobanch*e Control with Imidazolinone Herbicides in Various Host Crops**

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Imidazolinone herbicides, acetolactate synthase-inhibitors, were used for pre-emergence and post-emergence weed control. Imazethapyr (Pursuit, 100 g/l, SL) was recommended for selective control of *Orobanch*e *crenata* in pea, but its selectivity for other crops was not sufficient. In screening other herbicides from the same group, we found that low rates of imazamethapyr (Cadre, 240 g/l, SL) efficiently controlled *Orobanch*e spp. and were relatively selective for some host crops. In field experiments, a split application of 2.5+5.0+5.0 g/ha imazamethapyr sprayed post-emergence on parsley plants, starting at the 4–6 leaves stage and followed on fresh sprouting after the first and the second harvests, prevented parasitism and did not affect yields, as checked in a noninfested location. In sunflower a split application of two portions of 2.5 g/ha imazamethapyr each post emergence, the

first when the crop reached the 4–6 leaves stage and the second ~2 weeks later, controlled *O. cumana* under dryland conditions. However, when irrigation was added, before crop blooming, more parasites appeared above ground, enough to damage yields. Tomatoes grown in *O. aegyptiaca*-infested fields were damaged by foliage application of imazamethapyr, 2.5–5.0 g/ha, which caused drop of flowers and early ripening fruits. Applying the same rates by chemigation in sprinkler irrigation led to the same damage but, after a few weeks there was heavy foliage growth as well as fruit setting, which was, however, too late for harvest. Potatoes were not damaged by three foliage applications of 2.5, 5.0 or 7.5 g/ha imazamethapyr, starting 2 weeks after crop emergence and continuing every 2 weeks. The infestation by *O. ramosa* in the experimental field was not heavy enough to measure the effect of the treatments on yields, in comparison with the untreated control.

#### **Isolation of Mycoherbicidal Pathogens from Juvenile Broomrape Plants**

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The parasitic broomrapes (*Orobanch* spp.) are widespread weeds, cutting yields in half on ca 4% of the world's crops. As these plants attach to crop roots, they cannot be controlled mechanically, except by removing their flower stalks after much of the damage to the crop and its yield has already been done. They are usually not amenable to selective herbicides (except those used postemergence), as most herbicides cannot differentiate between crop and parasite. Biological control could be an effective answer. The isolation of two highly parasitic fungi, *Fusarium arthrosporioides* strain E4a and *F. oxysporum* strain E1d, is reported from diseased, juvenile, emerging *Orobanch* flower stalks. Both organisms meet Koch's criteria for being primary pathogens. DNA RAPD fingerprinting showed that they are indeed different from each other and from many other *Fusarium* spp. as well as other *formae speciales* of *F. oxysporum*. Tomato plant roots dipped in the fungal suspension and planted in broomrape-infested soil were protected for long periods. There are still many experiments to be performed before agricultural use can be envisaged. These include studying the host-range of the pathogens on different *Orobanch* strains and species, soil residue analyses, pathogenicity on crops cultivated in the same geographical region to determine incompatibilities, as well as the development of cost-effective applications technologies. It is possible that basic studies of the defenses of broomrape will lead to synergies using chemical synergists to suppress defenses and/or facilitating microorganisms.

#### **Green Fluorescent Protein (gGFP) as a Marker in a Phytopathogenic Fungus, *Fusarium oxysporum*, on *Orobanch***

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*Orobanch* is a parasitic plant causing considerable damage to cultivated crops, primarily vegetables. Biological control using fungal pathogens as mycoherbicides is one part of an overall effort to put a halt to the infestation with *Orobanch* on crop plants. Basic studies of the biological interactions between *Orobanch* and its pathogenic fungi are needed to help find the most effective ways to increase the parasite's vulnerability and thus its control. Recently two fungal pathogens of *Orobanch aegyptiaca* were isolated and have shown promise as mycoherbicides. One aspect to be studied is the host–pathogen interaction, specifically the role of the pathogen in its encounter with the host. Additionally, there are interactions to follow between the pathogen and other microorganisms

which may be competitors. We have introduced the green fluorescent protein gene (gGFP) as a marker into one of the pathogens of *Orobanche*, viz., *Fusarium oxysporum*, as a means to be able to follow and study the host–pathogen interactions, and the pathogen–microbe interactions which occur in soil.

### **Grafting for *Orobanche* Resistance**

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Experiments that have been conducted during the last 10 years revealed full *Orobanche* resistance can be achieved in a susceptible crop by grafting on a resistant scion. We found differences in the behavior of host plants between grafts in which resistant hosts were used and those based on tolerant plants. Full resistance was achieved only with resistant rootstocks. In all experiments we compared infestation with *Orobanche* in non-grafted plants and in the following graft combinations: susceptible scion on resistant rootstock, resistant scion on susceptible rootstock, and self-grafting of either resistant/tolerant or susceptible plants. Graft survival was usually very high. No *Orobanche* developed on any of the grafts with resistant rootstocks. In this case the grafted plants showed no ill effects in spite of their growing in *Orobanche*-infested soil. When the resistant scions were grafted on susceptible rootstocks, they were attacked by *Orobanche* with typical symptoms. The use of tolerant genotypes gave different results. These plants conferred tolerance both as scions and as rootstocks. The resulting grafted plants were infested with broomrape, but to a lesser extent. This difference between resistance and tolerance can be explained by the plant's response to the parasite. In contrast to the situation with the tolerant plants, the reaction in the resistant plants is a local hypersensitive response in the roots. Grafting susceptible varieties on resistant rootstocks may allow immediate use of desired genotypes that are susceptible to *Orobanche* in infested fields, thereby providing an interim solution until new resistant varieties are developed.

### **Effects of Herbicide Inhibitors of Carotenoid Biosynthesis on Field Dodder (*Cuscuta campestris*)**

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Field dodder (*Cuscuta campestris*) is an obligate shoot parasitic weed, which causes damages and yield losses in various crops worldwide. The yellow-orange dodder shoot is characterized by a high content of carotenoids, which mask the small amount of chlorophylls and play an important role in protecting the plant from photo-oxidative damages.

The effects of post-emergence application of three herbicides inhibiting carotenoid biosynthesis were tested on dodder parasitizing carrot plants. Flurochloridone ('Racer' 250 g/l, E.C.) at 500 g a.i./ha, and two triketones – sulcotrione (52% soluble granules) at 250 g/ha and ZA-1296 (488 g/l, S.C.) at 250 g/ha – were examined. These herbicides inhibit directly (flurochloridone) or indirectly (triketones) the activity of phytoene desaturase, a key enzyme in the carotenoid biosynthetic pathway, characterized by bleaching of the treated tissue. Quantitative analysis of carotenoid precursors –  $\beta$ -carotene and phytoene – in the stem was used to determine the effect of these herbicides on field dodder. The effect of flurochloridone on dodder was rapid; 8 h after application,  $\beta$ -carotene content was decreased, with a simultaneous increase in phytoene level. Hence, 48 h after treatment the bleached stem contained only 2–16% of the initial  $\beta$ -carotene level. The effect of the triketones was similar but slower and all the  $\beta$ -carotene disappeared 6 days after treatment. Recovery of the  $\beta$ -carotene content in the shoot tip was evident 4 days after flurochloridone treatment. An intermediate

zone, rich in  $\beta$ -carotene, developed 6 days after treatment between the orange shoot tip and the bleached region on the stem. No recovery was observed 6 days after triketones treatment, and the developing stem was entirely bleached, with no  $\beta$ -carotene and a high content of phytoene. In spite of the effect on carotenoid biosynthesis, none of the three herbicides affected dodder stem elongation.

All treatments led to histological changes in the dodder stem with massive destruction of amyloplasts in the parenchyma cells within the cortex and the pith tissue. Concomitantly, a sharp depletion in starch and soluble carbohydrate content was observed. On the whole plant level, the herbicides prevented the formation of attachments and biomass accumulation in dodder, with flurochloridone being less active than the triketones. The development of reproductive organs (buds, flowers and fruits) was repressed by sulcotrione and ZA-1296, but not with flurochloridone. In this study, the triketones exhibited better herbicidal activity than flurochloridone in controlling field dodder in carrots, but their selectivity to the crop was lower.

C: NEW APPROACHES TO WEED CONTROL

### **Treatment of Transgenic Herbicide-Resistant Seeds for Broomrape Control**

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Parasitic weeds cause extensive damage to many crops. Experiments were designed to overcome three problems: (i) damage caused by parasitic, root-attaching weeds that can be killed by systemic herbicides; (ii) contamination of crop seeds with small numbers of weed seeds; and (iii) cost of off-target spraying of herbicides. We addressed these problems by using target-site herbicide-resistant crops that can allow crop seed treatment with relatively concentrated herbicides. Loading glyphosate- and imazapyr-resistant crop seeds with their respective herbicides prevented infection of the emerging crop by *Orobanche*. Dry glyphosate-resistant and imazapyr-resistant rape seeds were allowed to imbibe in glyphosate or imazapyr solutions, respectively. The seeds were then sown in broomrape-infested soil. Under the experimental conditions in these pot experiments, treating rape seeds with 0.7 M glyphosate or 0.1 M imazapyr was optimal for *Orobanche* control. These seed treatments completely prevented parasitism by broomrape and allowed normal growth of the rapeseed plants with no broomrape damage. Soaking seeds of various weeds (e.g. *Phalaris minor*, *Avena sterilis*, *Amaranthus retroflexus*) in glyphosate under similar conditions, resulted in failure of the emerging weed seedlings to establish and survive. Such treatments may selectively control contaminating weed seeds in seed stocks of herbicide-resistant crops.

### **Dryland Field Crop Rotation for Integrated Control of Weeds and Other Pests**

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The effect of a dryland 6-year crop rotation vs. monocropping with wheat was compared for managing weeds and other pests at three locations. In a loose soil at Be'eri in the Negev, the treatments which are compared with monocropping of wheat include a rotation with 2/3 wheat, i.e., a broadleaved crop is planted following 2 years of wheat; rotation with half wheat, i.e., wheat and a broadleaved crop are planted alternately (1/2); and a rotation with wheat every 3 years (1/3). The field that was free of broadleaved weeds and wild grasses in 1994, was heavily infested in 1998 with both and needed an expensive mixture of herbicides for their control, in addition to chemical

broadleaves control in 1997. Wheat in rotation (1/3) was only slightly infested with grasses, and below the threshold for necessitating chemical control. Dr. D. Orion (Dept. of Nematology, ARO, The Volcani Center, Bet Dagan) identified soil nematodes infesting mainly the monoculture wheat plots, causing significant yield reduction in this treatment. In medium clay soil at Gesher HaZiv, three 6-year rotations in addition to wheat every third year, vetch, chickpeas, watermelon and corn for silage were established, and compared with a monoculture of wheat. Wheat infestation with various wild grasses increased heavily in the monoculture, compared with the other treatments; evaluation of wheat vigor showed that in monoculture the crop suffers from other soilborne pests which will be identified this season. On heavy clay soil at Neve Ya'ar, the crop rotation includes two wheat crops, for grain and for silage, alternating with vetch, chickpeas, sunflower or corn for silage. Winter wild grasses infestation increased heavily in the monocultured wheat, compared with the wheat in rotation, but to date no other weed pests have been identified. Grain yields of the monoculture wheat which was treated with selective grass killers were good.

### **Subsurface Drip Irrigation Can Reduce Herbicides Use in Cotton**

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In subsurface drip irrigation (SUDI), the tubes and drippers are buried at a depth of 40–50 cm. This way water and nutrients are provided directly to the plant roots, while the soil surface remains dry throughout the growth season, and seed germination is prevented. The lower amount of weed infestation reduces the need for chemical weed control. On the other hand, the absence of moisture in the upper layer of the soil prevents the microbial degradation of herbicides, which results in herbicide carryover and may cause damage to the following crop. Field trials were conducted during the 1996–97 seasons in order to examine the weed infestation, herbicide fate and performance of cotton grown under SUDI vs regular surface drip irrigation (SDI). Soil samples were taken at different times after application and herbicide residues were determined by chemical analyses and bioassay. The lack of moisture in the top layer of soil resulted in a lower weed infestation in SUDI as compared with SDI plots, both with and without herbicides. We tested the fate of herbicides that were applied in early spring, using bioassays and chemical analyses. Wheat, planted as a sensitive test plant following the cotton crop, was injured more severely in SUDI plots – particularly those treated with trifluralin – than in SDI plots. Excessive irrigation given to SUDI cotton plots at the end of the cotton irrigation season (early September), which moistened the upper layer of soil, prevented this damage and increased wheat yield, as compared with wheat planted in SDI plots. Apparently, it is possible to grow cotton and other summer crops under SUDI conditions with minimal use of herbicides.

### **Cover Crops as a Means for Lowering Weed Infestation in Cotton**

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The use of cover crops to reduce the need for chemical control of weeds and insects and to improve soil structure is a well established practice throughout the world. Early studies conducted in Israel by Y. Kleifeld (Dept. of Weed Science, ARO, Israel) and co-workers have demonstrated the feasibility of various cover crops. A series of field experiments was conducted during 1995–97 in Kibbutz Revadim (coastal plain) on clay-loam soil. The effect of winter cover crops – purple vetch (*Vicia atropurpurea*), wheat (cv. 'Yaniv') and green pea (cv. 'Sunfrost') – on the level of

weed infestation and growth of cotton planted in the spring, was examined. An additional treatment consisted of mulching the soil surface with 'imported' wheat straw. The cover crops were cut in the spring and the shoot biomass was left to dry on the soil surface as mulch, to attract beneficial insects. The local chemical weed control practice (fluometuron 1.5 kg a.i./ha and trifluralin 1.0 kg a.i./ha soil incorporated in February, followed by prometryn 2.0 kg/ha pre-emergence) served as the control. Cotton was planted in the spring in a 'one-skip' design (one in four rows is skipped). Cover crops reduced the number of weeds significantly, with pea and vetch left on the surface as mulch being more effective than wheat mulch. Mulched plots were less infested with weeds than the treatments where the biomass was incorporated into the soil. Added wheat straw mulch was less effective than the other cut mulches, including wheat. The number of beneficial insects counted before the cover crops were cut was higher in pea and vetch than in wheat. No cotton yield reduction was observed in the cover crop treatments as compared with the chemical weed control. These data indicate that use of cover crops can be an efficient and cost-effective way to reduce the herbicide input in cotton.

### **Control of *Cyperus rotundus* by Improvement of the Biological Agent *Cercospora caricis* via Transformation with IAA and Cytokinins Genes**

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Purple nutsedge (*Cyperus rotundus*) is considered to be the world's worst weed, especially in the tropical and subtropical regions. Yield reductions may be as high as 80% in many crops. The rapid growth of purple nutsedge is aided by prolific propagation through a complex underground system of rhizomes and tubers. Its narrow leaves with thick cuticle make it extremely difficult to control by mechanical or chemical means. Some of the effective herbicides are not selective or do not provide a long residual effect; others exhibit poor translocation to the site of action in the dormant tubers. Biological control may offer a useful substitute for chemical herbicides and/or a means for integrated control. *Cercospora caricis* has been shown to have potential as a bioherbicide agent for purple nutsedge, but its efficacy must be improved in order to ensure consistently high levels of weed control. This may be achieved through genetic improvements in the capacity to produce phytohormones, e.g. IAA and cytokinins. Yogev and Rubin have reported that a combination of plant hormones applied postemergence twice a week for a period of 4 weeks, dramatically changed the purple nutsedge growth pattern: no new tubers developed on the rhizomes, which grew upward into basal bulbs, thus forming green shoots only. The objective of our research is to transform genetically a specific pathogen of purple nutsedge, *C. caricis*, with genes for phytohormones (IAA and cytokinins) production. We hypothesize that inoculation of purple nutsedge with the transformed fungi will prevent tuber formation by the host plant while accompanied by higher levels of disease incidence. We have succeeded to adapt and calibrate this approach in order to manipulate transformation in *C. caricis*. Recently we were able to express two genes in *C. caricis*,  $\beta$ -glucuronidase (GUS) under the GPD1 promoter of *Cochliobolus heterostrophus* as a reporter gene, and hygromycin B under the PtpC promoter of *Aspergillus nidulans* as a detectable marker.

### **Effect of Glyphosate Applied as a Preharvest Treatment on Cotton Plants and Weeds**

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Glyphosate in various formulations is widely used for non-selective weed control. Due to its high efficiency against annual and perennial weeds, broadleaves and grasses, and to its lack of soil activity, farmers use it for general weed control in many crops. During the last few years we have been testing this herbicide in cotton as a preharvest treatment for its activity as a plant growth regulator and for selective control of late germinating weeds. In field trials, we applied glyphosate as an isopropylamine salt (Roundup,<sup>R</sup> 360 g a.i./l) postemergence on cotton plants at different boll opening stages: start, 30–50%, and full boll opening. The herbicide was applied at different concentrations, either by air or ground sprayers. No injury symptoms were observed on the glyphosate-treated cotton plants at any of the times or rates applied. Germination and vigor of cotton seeds, however, were severely reduced, indicating translocation of the applied herbicide via the phloem to these organs, which operate as strong sinks. Similarly, seeds which developed on flowering weeds present at the time of application lost their germinability. This effect was observed particularly in wild poinsettia (*Euphorbia geniculata*), velevetleaf (*Abutilon theophrasti*), spurred anoda (*Anoda cristata*), and Palmer amaranth (*Amaranthus palmeri*). Significant detrimental effects were noted also on the germination of annual and perennial weeds. In most experiments, significant improvement was observed in the quality of the cotton yield and the farmer's income was increased. More experiments are now in progress in order to optimize the treatment in terms of glyphosate rate and application time.

### **Organo-Clay Formulations of Herbicides for Reducing Environmental Contamination**

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New formulations of alachlor and metolachlor were designed and tested by adsorbing the herbicide to montmorillonite clay particles, the surfaces of which were modified from hydrophilic to hydrophobic by pre-adsorption of organic cations such as benzyltrimethylammonium (BTMA) or benzyltriethylammonium (BTEA). Alachlor and metolachlor adsorption on clay-BTMA complexes was significantly higher than that observed on unmodified clay or clay pre-adsorbed with hexadecyltrimethylammonium (HDTMA). BTMA pre-adsorbed at a load of 0.5 mole/kg clay gave better formulations of alachlor and metolachlor than those pre-adsorbed with BTMA at the full cation exchange capacity (0.8 mole/kg). Slow release of the herbicides to the soil solution maintained the herbicidal activity in the top soil, as determined by a bioassay using *Setaria viridis* and wheat as test plants. Reduced leaching was also observed under field conditions in sandy soil. Laboratory and field experiments demonstrated that weed control is improved and extended when alachlor or metolachlor is formulated with organo-clay complexes. In addition, the organo-clay formulations were significantly better protected from photodegradation and volatilization. The environmental and agronomic applications of the new formulations were demonstrated.

*D: CHEMICAL WEED CONTROL*

### **Improving Annual Weed Control in Peanuts**

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Field experiments with peanuts in 1996 and 1997 were established on loose soil in the Negev and on mixed peat soils in the Hula Valley, in an attempt to improve selective weed control. In

1996 we found that 50 g/ha flumioxazin (Strike, 50%, WP) applied pre-emergence was selective for peanuts, and adding it to terbutryn (Terbutrex, 500 g/l, SC) or oxifluorfen (Goal, 238 g/l, EC or Galigan, 240 g/l, SC) improved control of pigweed (*Amaranthus* spp.), including *A. blitoides*. In 1997, new herbicides were tested pre-emergence: oxadiargyl (Raft, 400 g/l, SC), ready mixtures of fluthiamide with metribuzin (24%+17.5%, respectively; and Axiom 54%+14%, respectively, WG), and imazethapyr (Pursuit, 100 g/l, SC). The herbicides showed good crop selectivity, but were evaluated for weed control only in the Hula, since the experimental field in the Negev was free of weeds. The herbicides effectively controlled pigweed, common purslane (*Portulaca oleracea*) and velvetleaf (*Abutilon theophrasti*). The same held true for a new formulation of metolachlor (Dual Gold, 832 g/l, EC) which was mixed with terbutryne. In a field that had not been treated pre-emergence, postemergence application of imazamethapyr (Cadre, 240 g/l, EC) halted purslane growth, but no control was achieved. Effective growth inhibition of morning glory (*Ipomea* spp.) was obtained by postemergence application of pyridate (Lantagran, 640 g/l, EC) mixed with fomesafen (Flex, 240 g/l, EC). Wheat, corn, sunflower and cotton planted in soil samples that were taken after crop harvesting from the peanut plots that had been treated with imazethapyr, 80 g/ha, in the loose soil, were not damaged. Wheat and sunflower planted in soil samples from the mixed peat soil which was treated previously with imazamethapyr, were not affected by the herbicide's residual effect, but cotton and corn were damaged; the typical growth retardation caused by herbicide residue vanished after a couple of weeks.

### **Selective Control of Annual Weeds in Direct Seeded Paprika**

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Paprika emergence and early development is very slow and during that period the crop is very sensitive to weed competition. An effective herbicide for preplanting or pre-emergence application in this crop is essential, since the recommended herbicides isopropalin (Paarlan, 720 g/l, EC) and napropamide (Devrinol, 450 g/l, SC), which have been used for that purpose, are no longer sold in Israel. In field experiments established in 1997, we found that the herbicide clomazone (Command, 315 g/l, SC), applied preplanting and incorporated mechanically or pre-emergence followed by sprinkler irrigation, showed good crop selectivity in sandy and loose soils in the Negev and medium clay soil in the Golan heights. Clomazone partly controlled pigweed (*Amaranthus* spp.), and was very effective in control of heliotrope (*Heliotropium* spp.) when mechanically incorporated into the soil. The herbicide oryzalin (Surflan, 500 g/l, SC) heavily damaged paprika when soil incorporated, but was found safe when applied pre-emergence. Oryzalin effectively controlled pigweed in the clay soil, but in the lighter soils left some pigweed and heliotrope. The herbicide aclonifen (Challenge, 600 g/l, SC), which was recommended for pre-emergence application in paprika but later canceled because it caused severe damage in some fields, was tested at all experimental locations and did not damage the crop. The herbicide was tested at 1.5 kg/ha on light soils and 1.8 kg/ha on clay soil, and was effective in pigweed control but not in heliotrope. The field experiments suggest selective weed control in paprika, but better weed control could be achieved by mixtures of herbicides which promise a broader spectrum of control and prevent escape of some weed species.

### **Command for Weed Control in Paprika at Dvir, 1997**

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The product 'Command' (clomazone, capsulated suspension, 360 g a.i./l) was examined by two methods of application, preplant incorporation (PPI) or pre-emergence application (PEA), and at

different rates for weed control in paprika (Dvir, 1997). The dates were 23.III.97 – PPI; 6.IV.97 – sowing; 7.IV.97 – PEA. The dominant weeds in the trial area were *Amaranthus album*, *A. blitoides*, *Heliotropium* spp. and *Solanum nigrum*.

Conclusions: (i) Command, at PEA, was very effective in controlling weeds in paprika. There is no need to apply the product in PPI. (ii) There were no significant differences among the various treatments; all treatments differed significantly from the untreated. (iii) Command was not phytotoxic to paprika. (iv) There were no significant differences in the yields of all treatments.

The results of the treatments were as follows: No. 1, 'Command', PPI at 1.5 l/ha - 2% a\* weed infestation; No. 2, PPI at 2.25 l/ha - 2% a; No. 3, PPI at 3 l/ha - 0% a. No. 4, 'Command', PEA at 1.25 l/ha - 5% a; No. 5, PEA at 1.5 l/ha - 0% a; No. 6, PEA at 2.25% l/ha - 0% a. No. 7, 'Enide' (diphenamide, wettable powder, 500 g a.i./kg), PEA at 6 kg/ha - 6% a. No. 8, 'Paarlan' (isopropalin, emulsifiable concentrate, 720 g a.i./l), PPI at 2.5 l/ha - 2% a. No. 9, Control, 15% b. [\*Numbers followed by the same letter do not differ significantly,  $P < 0.05$ .]

### **Weed Control in *Hypericum* spp. at Kibbutz Nirim, 1996/97**

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*Trial I:* *Hypericum* spp. cv. 'Excellent Flair' was pruned on 21.XII.96 and the treatments were sprayed 3 days later. Harvest for export was on 20.VI.97. *Trial II:* A field of *Hypericum* spp. cv. 'Rhinegold' was first pruned on 4.III.97 and the treatments were sprayed 7 days later. The branches were 15–20 cm long. Harvest for export was on 29.VII.97. The field was pruned again on 29.VII.98 and the treatments were sprayed 10 days later. Harvest for export was on 19.XII.97. In all trials the treatments were incorporated via sprinkler irrigation of 20 mm. The dominant weed species in the field were *Sonchus oleraceus*, *Conyza* spp., *Cyperus rotundus* and *Convolvulus* spp. The treatments were: (i) Eptam (EPTC 760 g/l, EC), 10 l/ha; Triflurex (trifluralin 480 g/l, EC), 2.5 l/ha; Terbutrex (terbutryne 500 g/l, SC), 2.0 l/ha; Alapaz (alachlor 480 g/l, EC), 5.0 l/ha; and untreated control. No phytotoxicity was observed in the crop and the flowers were of export quality. The Terbutrex 2.0 l/ha treatment applied after pruning or on branches of up to 20 cm length gave the best weed control over the broadest spectrum of weed species.

### **Weed Control in Ornamentals and Flowers**

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More than 20 species of flowers and ornamentals were checked for herbicides tolerance in an attempt to achieve efficient weed control in the crops in fields and greenhouses. The first step was screening of various herbicides on these crops grown in small pots; treatments that were successful were then applied in commercial fields or greenhouses to confirm the early results. The main difficulties in weed control in these crops arise from the frequent introduction of new ornamental species, the diversity in their cultural conditions and the high sensitivity of the crops to herbicides. In Israeli greenhouses, the worst weeds in the ornamentals are *Conyza* spp., *Cardamine hirsuta*, *Euphorbia* spp. and *Stellaria media*. In the open field, the worst are: *Amaranthus* spp., *Daucus* spp., *Silybum marianum*, *Solanum nigrum*, and many grass weeds. *Calicarpa* showed tolerance to simazine, pendimethalin and diuron; *Eucalyptus* tolerated simazine, oxyfluorfen, terbuthylazine; *Photinia* tolerated simazine diuron, terbuthylazine and pendimethalin; *Adenanthos* tolerated simazine, pendimethalin and terbuthylazine; *Pyttosporum* was found tolerant

to isoxaben, aclonifen and oxadiargyl; *Leocadendron* (Safari) tolerated oxyfluorfen, terbutryn and thiazopyr. *Acadia baileyana* tolerated oxadiargyl, pyridate and neburon; *Cestrum* tolerated simazine, pendimethalin, diuron and terbuthylazine; *Anemone* tolerated postemergence treatments with alachlor, aclonifen and acetochlor; *Ranunculus* showed some tolerance to low rates of terbacil; *Liatris* tolerated pre-emergence treatments with trifluralin, dinitramine, pendimethalin and neburon, and postemergence treatments with pyridate and clopyralid; *Lilium* tolerated diuron, oxadiazon and pendimethalin; *Eremurus* tolerated simazine, diuron, pendimethalin, aclonifen and terbacil; *Anigozanthus* tolerated dimethenamid, metolachlor, pendimethalin, and the granular formulation of isoxaben + trifluralin; *Asparagus virgatus*, *A. meyerie* and *A. myricladuaes* tolerated propyzamide, low rates of simazine and terbacil, but selectivity varied with the growth season; *Solidago* tolerated propyzamide and isoxaben; *Helianthus annuus* tolerated alachlor, acetochlor, imazamethabenzmethyl, oxadiazon and aclonifen; *Aster* tolerated oxyfluorfen, oxadiargyl, oxadiazon and aclonifen.

### **Derby for Postemergence Broadleaves Control in Wheat**

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Derby S.C. is a broadleaved weed controlling agent in wheat. Derby is a trademark of Dow AgroSciences, containing flumetsulam 100 g/l + florasulam 75 g/l. The product is one of the triazolopyrimidines, and acts as an ALS inhibitor. It is a postemergence broadleaved weeds controller, and is absorbed through leaves and roots. Derby at 60 ml/ha controls the Cruciferae *Ammi majus*, *Anthemis pseudocotula*, *Astragalus palaestinus*, *Beta vulgaris*, *Cichorium pumilum*, *Chrysanthemum coronarium*, *Chrysanthemum segetum*, *Emex spinosa*, *Fumaria parviflora*, *Lavatera* spp., *Malva nicaeensis*, *Ranunculus arvensis*, *Senecio vernalis*, *Trifolium* spp., and sunflower volunteers. In order to control *Carthamus tenuis*, *Centaurea iberica*, *Silybum marianum* and *Notobasis syriaca*, Derby 50 mg/ha should be tank-mixed with 250–500 ml/ha bromoxynil, 500–1000 ml/ha MCP-P or 500–1000 ml/ha 2,4-D. Combination with foliar fertilizers, uran and urea did not increase the fertilizers' phytotoxicity in comparison with phytotoxicity of these fertilizers alone. In order to control broadleaved weeds and *Phalaris paradoxa*, *Ph. brachystachys*, *Lolium rigidum* and *Avena sterilis*, Derby should be tank-mixed with cloquintocet + clodinafop-propargyl (Topik) 600 ml/ha. Tank-mix of Derby with fenoxaprop (Puma Super) reduces the efficacy of *Phalaris* spp. control by Puma Super. The following crops can be planted 4 months after spraying Derby: corn, tomato seedlings, cotton and sunflower; and carrot and beet can be planted 6 months after spraying.

### **Azafenidin – A New Pre-emergence Herbicide in Orchards**

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Azafenidin (Evolus, 80% a.i./kg, WG) is a new pre-emergence herbicide (manufactured by Du Pont de Nemours Agricultural Products), designed for selective control of annual grasses and broadleaved weeds in grapes, olives, citrus, and deciduous and subtropical trees. Azafenidin, activated by rainfall or sprinkler irrigation, is absorbed mainly through the shoots of the emerging susceptible weeds and has weak mobility in the phloem or xylem. It inhibits the porphyrin biosynthetic pathway, resulting in cell membrane disruption. Azafenidin features low water solubility (18 ppm) and strong binding to soil and has low mobility in most types of soils. When applied postemergence, Azafenidin scorches young seedlings of susceptible weeds. The postemergence activity is enhanced when a surfactant is added. Azafenidin (240–480 g a.i./ha) applied pre-emergence, in autumn-winter and/or spring application in young groves, was found to be highly effective in controlling a wide range of annual grasses and broadleaved weeds for 3 and 5 months,

respectively. The following weed species were found susceptible to azafenidin: *Phalaris* spp., *Avena sterilis*, *Lolium rigidum*, *Digitaria sanguinalis*, *Lamium* spp., *Sonchus oleraceus*, *Lactuca seriola*, *Senecio vulgaris*, *Sinapis* spp., *Erucaria* spp., *Capsella bursa-pastoris*, *Beta vulgaris*, *Urtica* spp., *Malva* spp., *Ammi visnaga*, *A. majus*, *Plantago lagopus*, *Solanum nigrum*, *Portulaca oleracea* and *Amaranthus* spp. However, in several trials only limited or short-term activity was observed on the following weeds: *Astragalus* spp., *Medicago* spp., *Lupinus* spp., *Stellaria media*, *Conyza* spp. and *Amaranthus blitoides*, especially when low rates were applied. In order to improve and extend the range of weed control, azafenidin can be tank-mixed with reduced rates of other recommended pre-emergence herbicides, such as simazine, terbutryn or diuron. Azafenidin was found to be very safe (at rates as high as 800 g a.i./ha) when sprayed in two consecutive years on young trees, starting from the first year of planting. So far, a high degree of selectivity has been found in grapefruit, pomelo, orange, mandarin, peach, prune, nectarine, apple, pear, avocado and olive trees and grape vines. Selectivity tests will be continued in order to complete a period of three consecutive years.

### **Control of Winter Annual Weeds with Imidazolinone Group Herbicides, and Glyphosate**

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In recent years imazapyr weed killer has been included in the regular roadside maintenance program, for the control of winter annual weeds, as well as of a few perennials, e.g. *Hypparrhenia hirta* and *Desmostachya bipinnata*. However, since roadsides are occasionally adjacent to desirable vegetation such as orchards, vineyards, etc., which have manifested in the past varying degrees of susceptibility to imazapyr, it was necessary to conduct further trials with different herbicides having, basically, the common property of being more selective towards, and hence more suitable for use in close proximity to, such vulnerable vegetation. The relatively new compound imazapic, which has already been approved (in the USA) for use in peanuts (under the trade name Cadre) and along roadsides (under the trade name Plateau), was chosen for the 1996–97 experimental program. The trials were carried out along four highways in different regions of the country and consisted of the following treatments: imazapyr at 1000 g/ha + glyphosate at 1800 g/ha as the standard treatments; imazapic at 75 g/ha; imazapic at 37.5 g/ha + glyphosate at 1800 g/ha; and imazapic at 75 g/ha + glyphosate at 1800 g/ha. The vegetation at the time of treatment consisted of winter annuals, 30–50 cm high, of the Compositae, Cruciferae, Malvaceae and Gramineae families.

### **Chemical Weed Control in Drainage Canals in the Hula Valley**

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The drainage canals in the Hula Valley are used to control the groundwater table and remove excess water from the agricultural land near Lake Kinneret (Sea of Galilee). The canal system was restored recently for halting the peat oxidation and preventing the drainage of toxicants into the national water reservoir. The canals conduct water and are therefore wet the year round and were also used for pumping water into the irrigation pipes. Weed infestation inhibits canal functioning and mechanical weed control is expensive because the vegetation recovers quickly.

In studying chemical weed control in the canals, which consist of peat soil, we divided the canal into three portions according to its function, topography and weed population: The river bed, 2–3 m wide, covered with water, infested with the floating weeds *Photamogeton* and *Ceratophyllum*, as well as with *Phragmites*, *Typha*, *Polygonum* and *Paspalum*, which enter from the edges; the slopes, infested with *Cyperus rotundus*, *Cynodon dactylon* and, during the winter, with *Conyza* spp. and *Malva*; and the shoulders on both sides, infested with annuals and perennials typical to the neighboring fields. A

treatment with imazapyr (Arsenal, 250 g/l, SL) at 2.5 kg/ha applied on the river bed when the water stream was stopped and the water level dropped, controlled the weeds in this band and prevented its recovery for several months. Furthermore, the herbicide was not detected by chemical analysis or by bioassay in the water after the flow was returned. The untreated controls were definitely constant. The imazapyr-treated plots were covered by *Photamogeton* and *Ceratophyllum* in the summer. The weeds were left to protect the slopes from erosion during the winter. In March, glyphosate (Roundup 480 g/l, SL), 1.8 kg/ha with additives, was applied. The most active additives were flumioxazin (Strike, 50%, WP) at 0.125 kg/ha, oxifluorfen (Goal, 238 g/l, EC) at 0.125 kg/ha or imazamethapyr (Cadre, 240 g/l, SL) at 0.125 kg/ha. Late spring rains caused germination of summer weeds on the slopes, but the addition of imazamethapyr or imazapyr, at 1.25 kg/ha, prevented infestation with summer broadleaves and grasses. Imazamethapyr did not control *Conyza* spp. Excellent annual and perennial weed control, including *C. rotundus*, was achieved on the shoulders by autumn treatments with imazapyr+imazamethapyr, 1.25+0.06 kg/ha, or imazamethapyr at 1.25 kg/ha.

#### E: RESISTANCE TO HERBICIDES

### Polymorphism in Plantain (*Plantago lagopus*) Populations Resistant to Herbicides

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Triazine-resistant populations of plantain (*Plantago lagopus*) were first identified in orchards and roadsides in the early 1990s. Following repeated use of simazine and bromacil in orchards for more than 15 years, a modified target site triazine resistance evolved. This resistance was characterized and confirmed at the whole plant level, in isolated chloroplasts and by fluorescence methods. Recently, we discovered two additional plantain populations conferring resistance to other chemical groups acting at different sites. Diuron resistance was discovered in a peach grove at Kibbutz Dvir that had been treated repeatedly with diuron for the last 8 years. In spite of the fact that both diuron and triazine herbicides inhibit the electron transport in PSII, they apparently bind to different sites on the D1 protein. Hence, there is no cross-resistance and the Dvir population is triazine-sensitive. When diuron ( $10^{-4}$  M) was applied to excised leaves taken from Dvir and Rehovot (sensitive) plants, the PSII electron transport in both populations was completely inhibited, as determined by chlorophyll fluorescence decay measurements. However, the fluorescence signal in Dvir plants recovered rapidly, whereas in the Rehovot plants it did not recover. The recovery indicates that the herbicide is degraded or detoxified faster in the Dvir population than in that from Rehovot. The other resistant population was found in a non-cultivated area near Na'an, where sulfometuron, an acetolactate synthase (ALS) inhibitor, had been applied for at least 5 years. Plants from Na'an survived postemergence treatment with 10 g a.i./ha of sulfometuron, whereas all other populations did not survive even 2 g a.i./ha. We also found the resistant plantain in a nearby wheat field. Additional studies of the mechanism responsible for the resistance to sulfometuron and its response to other ALS inhibitors are in progress. The fact that *P. lagopus* is a plant that is usually associated with non-cultivated sites but is increasingly invading cultivated fields, and the evolution of resistance with multiple mechanisms, indicate its polymorphism and high adaptability to diverse growth conditions. Similar behavior was reported in the multiple-resistant *Lolium rigidum* from Australia and *Alopecurus myosuroides* in Europe.

### Distribution of ALS-Resistant Weeds in Israel: An Update

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Since our last report, the number and range of weed species conferring resistance to herbicide inhibitors of the enzyme ALS (acetolactate synthase), has been increasing steadily. Five broadleaved weeds were added to the list: *Conyza canadensis*, *C. bonariensis* were found at Ganot and Soreq; *Ammi visnaga* was found at Ganot with *Orobanche aegyptiaca* parasitizing on it; *Plantago lagopus* was found at Na'an; *Aspergula arvensis* was detected at Ramla; these are in addition to *Amaranthus blitoides*, *A. retroflexus* and field dodder (*Cuscuta campestris*), which have been reported previously. This field dodder population collected at Ganot was associated with ALS-resistant *A. blitoides*. At all locations the apparently resistant weeds survived pre-emergence treatments of various ALS inhibitors combined with PSII inhibitors, usually 75 g/ha of sulfometuron methyl and 2.5 kg/ha of simazine. In addition, we found grass weeds such as *Crypsis schoenoides* and *Panicum capillare* in sites heavily treated with sulfometuron-methyl in Ganot. On the Golan Heights and in Galilee, there were places with high infestations of *Rostaria cristata* following similar treatment. Some of these weeds were multiple-resistant and exhibited altered target site resistance to triazine herbicides as well as ALS resistance. The rapid evolution and wide distribution of multiple-resistant weeds should alert the farmers to the danger of their invasion into cultivated fields and of the urgent need for more careful planning of the weed management practices followed in non-agricultural sites.

### **Resistant Mechanism of Field Dodder (*Cuscuta campestris*) to ALS Inhibitors**

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Field dodder (*Cuscuta campestris*) is an obligate shoot parasitic weed which causes damage and yield loss to various crops worldwide. The parasite shoot is yellow, rootless, lacks cotyledons and leaves, and coils around the host's stem and penetrates the vascular tissue by haustoria. When attached to the host plant, the parasite is fully dependent on the host for the supply of water, minerals, amino acids and assimilates. Recently, we discovered at Ganot field dodder plants parasitizing *Amaranthus blitoides* plants that are resistant to herbicides known as inhibitors of the enzyme acetolactate synthase (ALS). The resistance mechanism of the host plant (*A. blitoides*) was due to an alteration of the target site, namely, ALS. ALS is a key enzyme catalyzing the first step in the biosynthesis of branched chain amino acids (valine, isoleucine and leucine). When the resistant host plants associated with dodder are grown in a soil containing high levels of an ALS inhibitor such as sulfometuron-methyl, the herbicide is absorbed and translocated by the host and should thus reach the parasite and kill it. The fact that the field dodder survived led us to presume that the parasite also is resistant to ALS inhibitors. *In vitro* studies have shown that strong ALS activity is present in *C. campestris* shoot extract, indicating that this parasite is able to synthesize branched chain amino acids independently of the host plant. ALS isolated from the R biotype of *C. campestris* conferred resistance to various sulfonylurea, imidazolinone, triazolo-pyrimidine and pyrimidinyl-thiobenzoate herbicides with an R/S ratio of 5, 6, 10 and 8, respectively. The results confirmed that the resistance to these inhibitors in *C. campestris* is similar to that found in *Amaranthus* spp. and is due to a modified target site. These data demonstrate the potential danger conferred by the herbicide-resistant parasite weeds, which are easily spread and invade cultivated crops.

### **Resistance to ALS Inhibitors is Not Associated with Reduced Ecological Fitness in *Amaranthus* spp.**

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The resistance to ALS inhibitors in *Amaranthus* spp. discovered in the early 1990s in Israel, is due to an alteration of the target site, acetolactate synthase (ALS). The survival and spread of the resistant plants are highly dependent on their ability to compete (ecological fitness) with the sensitive wild-type plants. In the absence of the selecting herbicide, lower ecological fitness will result in gradual dissipation of the resistant plants. Biotypes of *Amaranthus retroflexus* susceptible (SuS) and resistant (SuR) to sulfometuron were grown in the field at 400 plants/m<sup>2</sup> stand in five different ratios (100%S; 75%S/25%R; 50%S/50%R; 25%S/75%R; 100%R) in the absence of herbicide. A similar experiment was conducted with *Amaranthus blitoides*, which is both sulfometuron- and triazine-resistant (SuR+TR), as compared with a sulfometuron- and triazine-susceptible biotype (SuS+TS) and with a sulfometuron-susceptible and triazine-resistant one (SuS+TR). No differences in final individual plant weight and leaf area were detected between SuR and SuS plants of *A. retroflexus*, indicating in this case the absence of a 'growth penalty' due to the acquired resistance trait. On the other hand, plant weight and leaf area of the multiple-resistant *A. blitoides* (SuR+TR) were significantly lower than of the SuS+TS biotype. When the comparison was made between SuR+TR and SuS+TR, no differences were detected between the two biotypes in shoot biomass or leaf area. These data indicate that the trait conferring resistance to sulfonylurea herbicides in *A. retroflexus* and *A. blitoides* is not associated with a growth penalty. However, the low ecological fitness observed in the multiple-resistant *A. blitoides* (SuS+TR) is due to the presence of triazine resistance. The triazine-resistant biotype conferred lower fitness than the wild type. In this ecological niche, where most plants are triazine-resistant, the sulfonylurea-resistant plants had no advantage. This phenomenon may explain the low spread and limited distribution of the SuR+TR *A. blitoides* plants.

### **Rapid Detection Methods to Identify Grass Weeds Resistant to ACCase Inhibitor Herbicides**

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The extensive use aryloxyphenoxy propionic acid and cyclohexandione graminicides that inhibit the enzyme acetyl co-enzyme A carboxylase (ACCase), has led to an increase in the evolution of weed populations that are no longer controlled by these herbicides. To date, 13 grass species have evolved resistance to ACCase inhibitors in 11 different countries throughout the world. The phenomenon has major economic significance because of the large areas infested and the lack of alternative graminicides of different mode of action. In Israel, we discovered a *Phalaris minor* biotype that is 20-fold more resistant to fenoxaprop-ethyl both at the whole plant level and at the ACCase enzyme level as compared with the susceptible wild type. The common methods to confirm resistance to ACCase inhibitors include spraying plants under controlled conditions – which is not very accurate, and is a laborious and time-consuming method. The enzymatic tests are more reliable but demand expensive equipment, high expertise and the use of hazardous chemicals. Recently we have attempted to develop reliable, rapid, efficient and cheap methods in order to detect resistant biotypes of grass species to ACCase inhibitors. The following rapid methods were used recently to distinguish between the response of resistant and susceptible *P. minor* to fenoxaprop-ethyl:

- Inhibition of seed germination in the presence of fenoxaprop acid (2 mg/l);
- Inhibition of shoot elongation of seedlings by fenoxaprop acid (6 mg/l);
- Inhibition of root elongation of seedlings by fenoxaprop acid (0.5 mg/l);
- Inhibition of pollen grain germination by fenoxaprop acid (200 μM).

The seed tests produce results after 7 days, whereas the pollen grain test is completed within a few hours. With little modifications these methods can be adjusted to identify resistant biotypes of other species and/or to other ACCase inhibitors.

## **Oxidative Stress Induces Antioxidant Enzyme Protectants and Paraquat Resistance in *Conyza bonariensis***

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Understanding the various processes plants use to cope with oxidative stress is problematic in cases in which there is no intra-specific genetic variation to allow correlations. Oxidative stress tolerance in a paraquat-resistant *Conyza bonariensis* biotype has cross-resistance to other oxidative stresses such as SO<sub>2</sub>, photoinhibition, and other herbicides that generate active oxygen species. We found that oxidative stress resistance in 10-week-old plants is correlated with elevated constitutive levels of antioxidant enzymes, a phenomenon which is pleiotropically controlled by a single gene. Paraquat also induced higher levels of antioxidant enzymes: In 10-week-old resistant plants, pretreatment with 1 micromolar paraquat for 24 h differentially increased superoxide dismutase by 30%, ascorbate peroxidase by 50%, dehydroascorbate reductase by 20%, monodehydroascorbate reductase by 25%, glutathione peroxidase by 80%, and glutathione reductase by 120%. Measurements at different time points showed that the level of the antioxidant enzymes in resistant plants pretreated with 1 micromolar paraquat was raised within 6 h, peaked within 18–24 h, and returned to the base level within 36 h. Paraquat pretreatment could further increase transient paraquat resistance by 2.5–3.0 fold. The sensitive biotype showed neither oxidative resistance nor elevated levels of antioxidant enzymes. It appears that each of these antioxidant enzymes is part of a correlated antioxidative system which allows oxidative resistance in this species.