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ECOLOGY AND SUSTAINABLE CONTROL OF THE PARASITIC WEED – FIELD DODDER (*Cuscuta campestris*)

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Executive Summary

The aims of this project are to improve the management of field dodder (*Cuscuta campestris*) in major crops of Kazakhstan. Dodder specimens were monitored and collected in different regions of both countries in order to taxonomically identify those species which cause damage to crops and develop adequate measures to control them. In addition, field and laboratory experiments were established in both countries to investigate the role and efficacy of herbicides in the field dodder control on major crops and non-agricultural land. In both countries the major dodder species were identified as field dodder (*Cuscuta campestris* Yonker) and *C. monogyna* Vahl. In order to monitor and characterize more species, the search will continued and more regions will be included. A unique phenomenon of high degree of natural resistance to phloem mobile herbicides was discovered by the Israeli group that might influence the choice of herbicides to be used in the field. The mechanism of this natural resistance has not yet identified. Similar studies will be performed in Kazakhstan in order to confirm the global nature of the phenomenon. Various herbicides were examined under field conditions in alfalfa, sugar beet, and non-agricultural conditions. Mechanical control measures were compared to hand (manual) weeding, but none of them was comparable to the efficacy of manual weeding. Field and laboratory experiments to optimize the weed management practices will be conducted in various crops such as safflower. The search for a graduate student suitable for training in Israel will continue.

Section I.

A) Research Objectives

The general aim is to improve the management of field dodder (*Cuscuta campestris*) in major crops of Kazakhstan

Specific aims:

a. To determine which species of *Cuscuta* are present in Kazakhstan and Israel, their distribution, preferred host plants and natural enemies.
b. To establish suitable management measures against major species in large scale field experiments
c. To develop a manual suitable for farmer's use for identification, distribution, host selection and selective control of *Cuscuta*.

B) Research Accomplishments:

The Israeli Report:

Introduction:

Although 12 *Cuscuta* spp. are described in the Israeli flora (Feinbrun and Danin (1991) *Analytical Flora of Eretz Israel*, 1040 pp. CANA Publishing House, Ltd. Jerusalem, Israel), our survey conducted throughout the country revealed only two species: *Cuscuta campestris* - the major one that infests numerous annual and perennial weeds and crops and *C. monogyna* which parasitized trees in the northern part of the country.

In the course of our studies on the response of field dodder to herbicides we developed a new technique - 'seed bioassay in sand in a Petri dish' which enabled us to examine the response of the parasite without being associated with a host plant. The method is described in details in the paper published recently in: **Weed Research (2003) 43: 341-347 (see attached)**.

In addition, a new phenomenon regarding the response of dodder to glyphosate and other herbicides that inhibit the biosynthesis of amino acid (AABI) has been discovered and reported in the above mention paper: *C. campestris* was found to be much more resistant to all AABI herbicides tested. The I_{50} value of *C. campestris* growth inhibition by glyphosate was eightfold higher than that of transgenic, glyphosate-resistant cotton (RR-cotton). The I_{50} value for *C. campestris* shoot growth inhibition by sulfometuron was above 500 μM, whereas that of sorghum roots was only 0.004 μM.

Natural tolerance of *Cuscuta campestris* to herbicides inhibiting amino acid biosynthesis

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Summary

The response of *Cuscuta campestris* Yuncker, a non-specific above-ground holoparasite, to amino acid biosynthesis inhibitor (AABI) herbicides, was compared with other resistant and sensitive plants in dose–response assays carried out in Petri dishes. *Cuscuta campestris* was found to be much more resistant to all AABI herbicides tested. The I_{50} value of *C. campestris* growth inhibition by glyphosate was eightfold higher than that of transgenic, glyphosate-resistant cotton (RR-cotton). The I_{50} value for *C. campestris* shoot growth inhibition by sulfometuron was above 500 μM, whereas that of sorghum roots was only 0.004 μM. *Cuscuta campestris* exposed to glyphosate gradually accumulated shikimate, confirming herbicide penetration into the parasite and interaction with an active form of the target enzyme of the herbicide, 5-enolpyruvylshikimate-3-phosphate synthase. More than half of the *C. campestris* plants associated with transgenic, glyphosate-resistant sugarbeet (RR-sugarbeet) treated with glyphosate or with transgenic, sulfometuron-resistant tomato (SuR-tomato) treated with sulfometuron recovered and resumed regular growth 20–30 days after treatment. New healthy stems developed, followed by normal flowering and seed setting. The results of the current study demonstrate the unique capacity of *C. campestris* to tolerate high rates of AABI. The mechanism of this phenomenon is yet to be elucidated.

Keywords: *Cuscuta campestris*, EPSPS, acetolactate synthase, transgenic crops, sulfometuron, glyphosate, herbicide tolerance.

Introduction

Cuscuta campestris Yuncker (field dodder), a member of the Convolvulaceae family, is a non-specific above-ground holoparasite, and as such is totally dependent on its host plant for assimilates, nutrients and water supply. Its wide geographical distribution and host range make *C. campestris* among the most damaging parasites worldwide, causing severe damages to carrots, alfalfa, sugarbeet, onions, legumes and other crops (Parker & Riches, 1993; Dawson *et al.*, 1994; Holm *et al.*, 1997). Unlike root parasites, *C. campestris* seeds do not require a specific stimulant to induce germination: mechanical or chemical scarification of the seed coat is sufficient to facilitate it (Hutchison & Ashton, 1980; Dawson *et al.*, 1994). The germinating seed emerges as a long, thin, rootless, yellow-orange leafless stem that coils around adjacent objects. When attached to the leaf or stem of a suitable host plant, thigmotropic responses and chemical recognition cause *C. campestris* to develop haustoria within a few days (Tsivion, 1979; Press *et al.*, 1990). This highly efficient absorption system allows the parasite to divert resources (water, amino acids and assimilates) from the host to itself (Tsivion, 1979; Dorr, 1987), thus reducing host vigour and dramatically lowering crop production (Press *et al.*, 1990; Dawson *et al.*, 1994).

Although all the organelles and metabolic mechanisms exist in *C. campestris*, an obligate parasite, many of them are not essential for its survival. This means that herbicides such as photosynthesis inhibitors will have no effect on it. However, amino acid biosynthesis inhibitors (AABI), such as glyphosate and acetolactate synthase (ALS) inhibitors, affect the growth of *C. campestris*. When applied to the host, these phloem-mobile herbicides accumulate selectively in the strong *C. campestris*

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sink and inhibit parasite growth (Fer, 1984; Liu & Fer, 1990; Bewick *et al.*, 1991; Nir *et al.*, 1996).

Glyphosate interferes with aromatic amino acid (phenylalanine, tyrosine and tryptophan) biosynthesis by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is a key enzyme in the shikimate pathway. Inhibition of the pathway leads to overproduction and accumulation of shikimate (Amerhein *et al.*, 1980; Geiger & Bestman, 1990). ALS inhibitors inhibit the synthesis of branched-chain amino acids (valine, isoleucine and leucine) by blocking the first step involved in the condensation of pyruvate (Ray, 1984). It is not yet clear what exactly causes the death of the plant, but there is evidence that both EPSPS and ALS inhibitors inhibit assimilate translocation within the plant (Bestman *et al.*, 1990; Hall & Devine, 1993; Kim & Vanden Born, 1996; Geiger *et al.*, 1999).

As an absolute parasite, when attached to a host, *C. campestris* operates as a 'super-sink' overcoming the host's sinks (Wolswinkel, 1984). The reported high herbicidal efficacy of AABI herbicides indicates that in spite of the amino acid supply from the host (Wolswinkel *et al.*, 1984), the parasite possesses its own host-independent amino acid biosynthesis pathways, which may conceivably be sensitive to these inhibitors. Root parasites, such as *Orobanche* spp. (Joel *et al.*, 1995) and *Striga* spp. (Kanampiu *et al.*, 2001), are effectively controlled when associated with a target-site-resistant host plant. In this study, *C. campestris* seedlings are shown to tolerate high AABI concentrations and recover from the herbicide application while growing on target-site-resistant hosts, indicating that these crops may not be a solution for the control of *C. campestris*.

Materials and methods

Plant material

Cuscuta campestris seeds were collected from the parasite growing on *Prosopis farcta* (Banks et Solander) Macbride in Or Haner (Northern Negev, Israel), cleaned and acid-scarified for 20 min before use. Glyphosate-resistant (RR) cotton (*Gossypium hirsutum* L., cv. DP5415RR), sugarbeet (*Beta vulgaris* L., cv. Pillar-RR) and soya bean [*Glycine max* (L.) Merr., cv. GL 2600RR] plants were used as the resistant reference for glyphosate treatments. Multiple-resistant seeds (to sulfonylurea and triazine herbicides) of *Amaranthus blitoides* S. Watson (Sibony & Rubin, 2003) collected from Ganot (Coastal Plain, Israel), and a dwarf tomato (Micro-Tom, *Lycopersicon esculentum* Mill.) transformed with the Ds378-GUS construct containing a sulfonylurea-resistant gene (SuR-tomato) (Meissner *et al.*, 1997) were used as target-site ALS-resistant plants, whereas wheat (*Triticum aestivum* L., cv. Ariel) was used as an enhanced-metabolism chlorsulfuron-resistant reference. Non-transgenic cotton (cv. DP5415), sorghum [*Sorghum bicolor* (L.) Moench cv. RS610, Hazera, Israel], sweetcorn (*Zea mays* L., cv. Jubilee), wheat (*T. aestivum*) and wild-type *A. blitoides* (collected from a field in Kfar Shmuel never treated with herbicides, Coastal Plain, Israel; Sibony & Rubin, 2003) were used as herbicide-susceptible controls.

Herbicides

Glyphosate (Roundup, 360 g a.i. L^{-1}, Monsanto) and several ALS-inhibitor herbicides were tested: sulfometuron-methyl (Oust, 750 g a.i. kg^{-1}, DuPont), chlorsulfuron (Glean, 750 g a.i. kg^{-1}, DuPont), rimsulfuron (Titus, 250 g a.i. kg^{-1}, DuPont), imazapyr (Arsenal, 250 g a.i. kg^{-1}, Cyanamid), imazethapyr (Pursuit, 200 g a.i. kg^{-1}, Cyanamid), flumetsulam (technical grade 800 g a.i. kg^{-1}, Dow Agroscience) and pyrithiobac-sodium (Staple, 850 g a.i. kg^{-1}, DuPont). Trifluralin (Trifluran, 48[illegible] g a.i. L[illegible] Agan, Ashdod, Israel), a known herbicide for *C. campestris* control (Dawson *et al.*, 1994) and a non-specific inhibitor of microtubule assembly, was used to test bioassay reliability.

Dose–response assays in Petri dishes

Dose–response assays were performed in 9-cm Petri dishes filled with thoroughly washed coarse sand (135 g) containing different concentrations of the herbicide (15 mL per dish) based on the method reported by Tal *et al.* (2000). *Cuscuta campestris* seeds were planted in the sand (without a host) 1 cm from the bottom of each dish. After sowing, the dishes were sealed and incubated in a dark room (28°C), tilted face up at an 80° angle. All other seeds were placed 2 cm from the top of the dish, and the dish was sealed and incubated face down under the same conditions. *Cuscuta campestris* shoot length was measured 4–5 days after sowing (DAS). The root lengths of all other species were measured 3–6 DAS, depending on the growth rate of each species.

Long-term response of hosts and parasites to herbicides

Experiments were conducted in a glasshouse under summer conditions (*ca.* 13-h days at 28°C and 22°C nights). Herbicides (commercially available formulations) were applied with a motorized laboratory chain-driven sprayer equipped with a flat-fan nozzle (8001E) at 245 kPa, calibrated to deliver 300 L ha^{-1}. SuR-tomato, RR-soya bean and RR-sugarbeet seeds (two to three per pot) were sown in pots (7 cm × 7 cm × 8 cm) containing

a potting mixture of sandy soil and peat (2:1, V/V) and 1 g kg^{-1} of a slow-release fertilizer (Osmocote 14-14-14 Scotts-Sierra Horticultural Products, Marysville, OH, USA). After emergence, the seedlings were thinned to one seedling per pot. *Cuscuta campestris* seeds (15–20) were sown near the stem of each host plant 2 weeks after sowing (WAS). Associated plants were ready for treatment 4 WAS. Pots containing *C. campestris*-infested RR-sugarbeet and RR-soya bean plants were treated with glyphosate, and pots containing *C. campestris*-infested SuR-tomatoes were treated with sulfometuron. Host and parasite development were monitored up to 8 weeks after treatment (WAT). At each assessment, the host plant and parasite were evaluated and scored from 0 (dead) to 5 (full vigour). After the last assessment, plant and *C. campestris* tissue were collected and separated for fresh and dry weight determinations.

Shikimic acid assay

Cuscuta campestris, sorghum and RR, and non-transgenic cotton seeds were sown in Petri dishes containing sand and different concentrations of glyphosate (5 μM to 100 mM). Seedlings (4 DAS) were removed from the sand, weighed and their shoot or root lengths were measured before freezing in liquid N_2 for the shikimic acid assay. Shikimate was extracted by grinding the tissue in HCl 0.25 M (1:5, tissue:solution), and its content was measured in a 10-μl aliquot of the supernatant according to Singh and Shaner (1998) and Cromartie and Polge (2000)

Statistical analysis

Dose–response assays were conducted in a completely randomized design with three to four replications. The means and standard errors were calculated for all assays. The log-logistic model (Seefeldt *et al.*, 1995) was used to analyse root and shoot elongation and fit dose–response curves; I_{50} values (herbicide concentration causing 50% growth inhibition) were calculated from the respective curves.

Results and discussion

Dose–response bioassays

Non-associated *C. campestris* tolerated much higher concentrations of AABI herbicides than all other plants tested (Table 1). The I_{50} value for inhibition of the parasite's initial growth by glyphosate was 8, 330 and 650-fold higher than the concentration required to inhibit RR-cotton, non-transgenic cotton and sorghum root growth, respectively (Fig. 1). Similarly, the sulfometuron I_{50} value for *C. campestris* shoot elongation inhibition was above 500 μM, whereas that required to inhibit sorghum root elongation was only 0.004 μM (Fig. 2).

To examine the possibility that the herbicides have different effects on germination and seedling development, sorghum and *C. campestris* seeds were first pre-germinated on wet filter paper, and then transferred to the sand-filled Petri dishes containing the herbicides. There was no difference in the response patterns and the I_{50} values were similar (data not shown).

Cuscuta campestris and sorghum seed development were similarly inhibited in the presence of 1 μM trifluralin, a microtubule-assembly inhibitor reported to strongly inhibit the germination and growth of *C. campestris* (Liu *et al.*, 1987; Dawson *et al.*, 1994; Nir *et al.*, 1996). The response of the parasite to low trifluralin concentrations established the reliability of the bioassay and confirmed that the tolerance of *C. campestris* to AABI herbicides is a unique reaction and not an artefact arising from the assay conditions. Furthermore, when other *C. campestris* populations from Israel (grown on *A. blitoides*) and California (grown on tomatoes) and a population of *C. monogyna*

Table 1 Response of *Cuscuta campestris* and other plants to herbicides applied in the sand-filled Petri dish bioassay. The log-logistic model (Seefeldt *et al.*, 1995) was used to calculate the I_{50} values from the dose–response curves of shoot (*C. campestris*) or root elongation

Herbicide	I_{50}							
	C. campestris	*Sorghum bicolor*	RR-cotton	Wheat	SuR-tomato	Maize	SuR-*A. blitoides*	SuS-*A. blitoides*
Glyphosate (mM)	52	0.08	6.2	–	–	–	–	–
Chlorsulfuron (μM)	86	0.72	–	1.1	–	0.014	–	–
Rimsulfuron (μM)	>1000	0.01	–	–	–	–	1.9	0.1
Sulfometuron (μM)	>500	0.004	–	–	480	–	–	–
Imazapyr (μM)	>1000	1.5	–	–	–	–	140	12
Imazethapyr (μM)	>1000	0.5	–	–	–	–	–	–
Flumetsulam (μM)	>1000	<0.001	–	–	[illegible]	–	–	–
Pyrithiobac (μM)	140	<0.001	–		250	–	–	–
Trifluralin (μM)	1	1	–	–	–	–	–	–

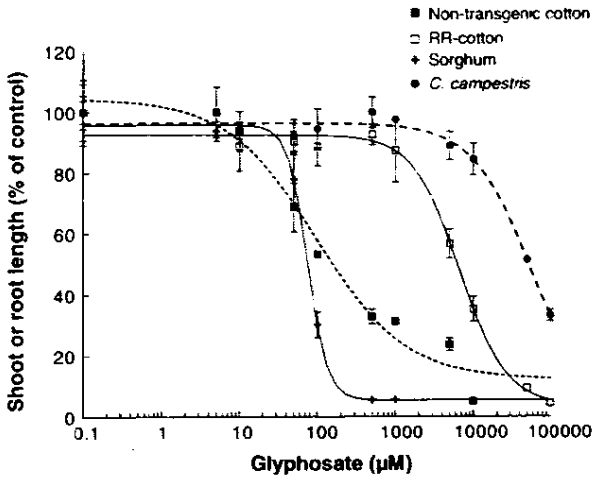

Fig. 1 Effect of glyphosate on the elongation of *Cuscuta campestris* shoots, and sorghum and cotton roots. The assay was conducted in sand-filled Petri dishes. Vertical bars represent SEM.

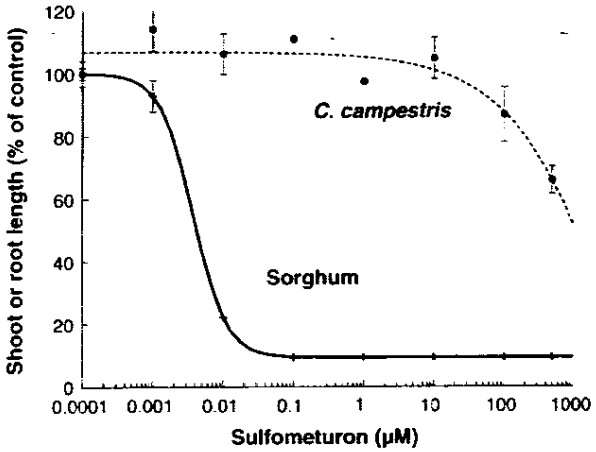

Fig. 2 Effect of sulfometuron on the elongation of *Cuscuta campestris* shoots (●) and sorghum roots (+). Vertical bars represent SEM.

from Israel (grown on *Ziziphus* spp.) were exposed to high glyphosate concentrations, a similar tolerance was observed (data not shown). Although a limited number of *Cuscuta* populations were examined, the results are more widely applicable.

Shikimic acid accumulation

Shikimate accumulation in plant tissue may be used as an indication of glyphosate inhibition of EPSPS (Singh & Shaner, 1998). The flow of shikimate through the shikimic pathway is disrupted by herbicide activity and therefore accumulates in the glyphosate-treated plant tissue (Geiger & Bestman, 1990). Only glyphosate concentrations above 1 mM reduced the growth of *C. campestris* shoot and RR-cotton root, whereas much lower concentrations were needed for non-transgenic cotton and sorghum root inhibition (Fig. 3). In both glyphosate-sensitive species, significant shikimate accumulation was observed at relatively low herbicide concentrations, whereas in RR-cotton virtually no shikimate accumulated at any of the concentrations tested, indicating that the observed growth inhibition in RR-cotton was not caused by EPSPS inhibition (Fig. 3). In *C. campestris*, however, glyphosate above 500 μM caused a gradual increase in shikimate accumulation, indicating herbicide penetration into the parasite and the presence of an active form of EPSPS (Fig. 3).

Response of host-associated C. campestris

Growth and development of *C. campestris* on herbicide-resistant host plants are inhibited soon after herbicide application, but after 10–14 days, many of the treated parasite plants recover and resume growth (Fig. 4). To estimate the ability of the parasite to recover from the herbicide treatment, its development was monitored over an extended period of time, up to 57 days after treatment (DAT).

Cuscuta campestris parasitism on herbicide-resistant host plants (RR-sugarbeet, RR-soya bean and SuR-tomato) resulted in severe growth retardation in the host plants (Table 2). Although post-emergence herbicide treatment (either glyphosate or sulfometuron) somewhat improved the host's biomass accumulation, it remained significantly lower than that of the untreated control (parasite- and herbicide-free hosts) and did not eliminate the damage caused by the parasite (Table 2). The parasite's response to the herbicide differed between hosts; most of the parasite plants growing on herbicide-treated RR-sugarbeet and SuR-tomato hosts survived, recovered and resumed regular growth (Table 2). The initial effect on the parasite was probably caused by the 'super sink' employed by the parasite, resulting in rapid translocation and accumulation of the herbicide in the parasite (Bewick *et al.*, 1991). Hence, soon after herbicide application, the parasite stems discontinued growth, turned deep orange and developed a deformed apex, but did not die. At a later stage, *C. campestris* plants recovered, the damaged apex resumed growth and developed broom-like shoots with three to five newly emerging stems which, 20–30 DAT, developed new healthy stems followed by normal flowering and seed set (Fig. 5).

Parasite recovery was not observed when associated with RR-soya bean. This was unrelated to herbicide treatment (Table 2), and probably due to a weaker association with the host as its stem became thick and rigid. Hence, *C. campestris* infestation on the soya bean

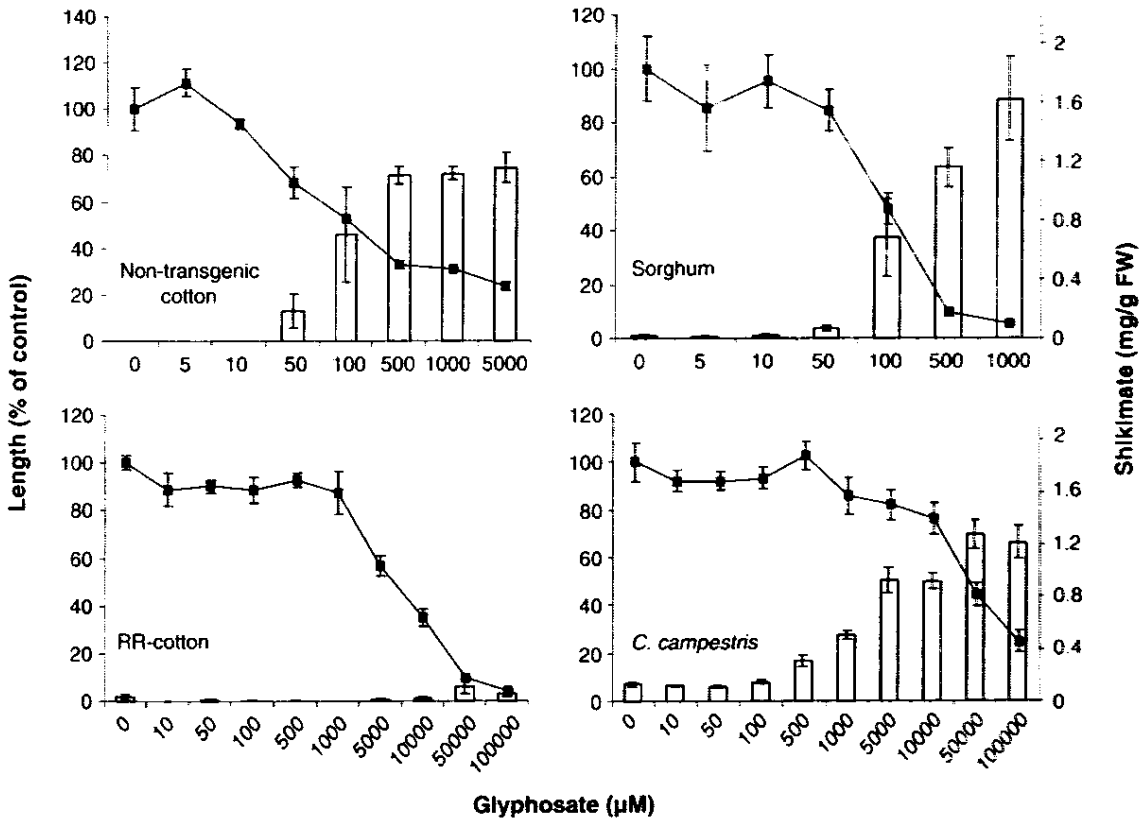

Fig. 3 Shikimic acid accumulation (bar) and root (cotton and sorghum) or shoot (*Cuscuta campestris*) length (line) at different glyphosate concentrations (µM). Vertical bars represent SEM.

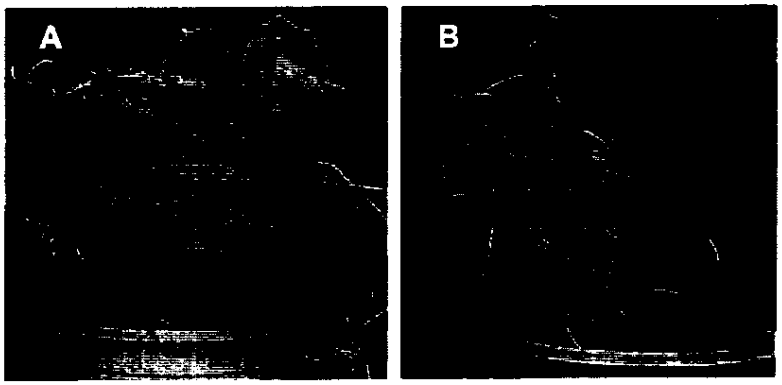

Fig. 4 RR-sugarbeet infested with *Cuscuta campestris* 30 days after treatment, untreated (A) and glyphosate-treated (1.08 kg a.e. ha^{-1}) (B).

plants declined shortly after the first visual assessment and by 57 DAT, all but one *C. campestris* plant were dead.

Although *C. campestris* is capable of parasitizing a large variety of hosts, and could be devastating to some crops, including soya bean (Table 2), incompatibility may develop following initial and successful establishment of the parasite's haustoria, due to mechanical or chemical elimination by the host (Werner *et al.*, 2001).

However, sugarbeet proved to be extremely sensitive to *C. campestris* infestation: RR-sugarbeet plants infested with *C. campestris* and not treated with glyphosate exhibited a steady decline in growth followed by a similar reduction in parasite vigour (Fig. 4). Although SuR-tomato was not an ideal *C. campestris* host (due to its size and short life cycle), well-established *C. campestris* plants were able to survive sulfometuron application.

Dawson *et al.* (1994) reported the incomplete kill of *C. campestris* after sulfosate and glyphosate application; surviving haustoria regenerated new shoots and treatment had to be repeated to achieve sufficient control of the parasite.

Table 2 *Cuscuta campestris* and resistant host plant (RR-sugarbeet, SuR-tomato and RR-soya bean) development 57 days after post-emergence treatment

Host plant	Parasite	Herbicide	Host fresh wt g per plant	*C. campestris* Fresh wt g per plant	*C. campestris* Number of survivors
RR-sugarbeet†	–	–	56 ± 7.7	–	–
	+	–	6 ± 1.8	2.3 ± 0.73	7/10
	+	+	32 ± 6.5	6.2 ± 2.22	5/10
RR-soya bean†	–	–	19 ± 1.01	–	–
	+	–	11 ± 1.03	*	*
	+	+	13 ± 1.45	*	*
SuR-tomato‡	–	–	52 ± 6.2	–	–
	+	–	13 ± 5.7	0.5 ± 0.25	5/8
	+	+	25 ± 6.5	0.8 ± 0.41	4/9

Shown as mean ± SE.
*No *C. campestris* survivors.
†Treated with glyphosate (0.72 kg a.e. ha^{-1}).
‡Treated with sulfometuron (22.5 g a.i. ha^{-1}).

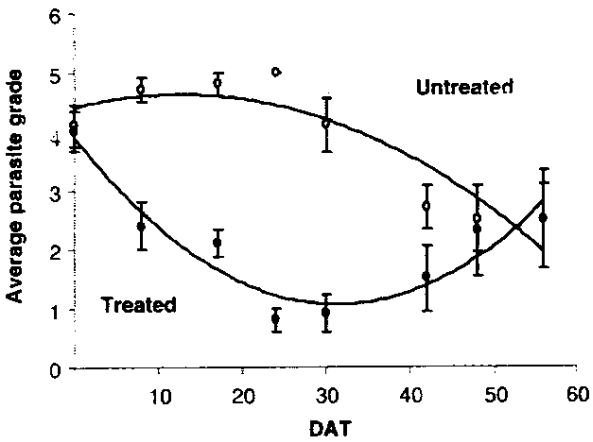

Fig. 5 *Cuscuta campestris* growth [(○) untreated; (●) treated] on RR-sugarbeet plants following post-emergence treatment with glyphosate (0.72 kg a.e. ha^{-1}). Parasite growth was visually evaluated and scored, with 0 representing no parasite growth, and 5 representing full parasite vigour and host coverage. DAT: days after treatment. Vertical bars represent SEM.

The question, therefore, is how do *C. campestris* seedlings continue to develop in the presence of high glyphosate concentrations and what is the mechanism that enables glyphosate- or sulfometuron-treated *C. campestris* to recover from the herbicide treatment? Herbicide tolerance in plants may be achieved by an altered target site, over-expression of the target enzyme, high specific activity of the target enzyme or enhanced detoxification of the herbicide (Yuan *et al.*, 2002). Shikimate accumulation in the parasite following exposure to glyphosate at relatively low herbicide levels eliminates the possibility of altered EPSPS, as well as that of enhanced herbicide detoxification. The possibility of over-expressed EPSPS remains to be investigated. Preliminary studies show that ALS extracted from *C. campestris* exhibits both high specific activity and lower sensitivity to sulfometuron (data not shown); this may indicate high RNA or protein expression of the target enzymes in *C. campestris* tissue.

The results of this study demonstrate the ability of *C. campestris* to tolerate and recover from high rates of glyphosate and sulfometuron application. These results raise questions about the claim that herbicide-resistant crops might provide a novel means for controlling *C. campestris* (Joel *et al.*, 1995). This approach should be evaluated for each host individually, taking into consideration the host morphology and host–parasite type of association. High compatibility between the host and the parasite (e.g. sugarbeet) may lead to less efficient control.

Acknowledgement

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Kazakhstan Report 2002-2003

Introduction:

Cuscuta spp. (dodder) is widespread around the world parasitizing trees, shrubs as well as numerous annual and perennial crops and weeds. There are 19 species infesting more than 168000 ha in Kazakhstan and 12 in Israel with field dodder Cuscuta campestris being the most important one (Zharasov, 1999).

According to the data collected in Kazakhstan the best control management of dodder is based on rotation of crops, well-timed and careful soil cultivation combined with optimal time of sowing and use of herbicides.

During the first year of the project (starting May 2002) several field experiments were established in order examine several management practices which combined cultivation and chemical measures to control the parasite. It was too late to initiate the crop rotation trials and they will conducted in summers of 2003-5. A digital camera has been purchased and is used for taking photographs of the different species.

Tasks of the studies on the first year (2002-2003)

1. Monitor the infestation and define the identity, structure and number of field dodder in crops
2. Examine the response of field dodder to new herbicides in alfalfa and sugar beet in fields and in the laboratory.
3. Investigate the role and efficacy of herbicides in the field dodder control on non-agricultural land.

Methods

Field experiments were conducted in the following farms: 'Ray of the Orient', 'Kisanov' and 'Abdygulov', in 'Talgar' district of Almatinskii region (oblast). The soil is middle-loamy light chestnut, 1.9-2.1% organic matter, pH - 8.05. The analysis of the weather conditions for 2002 is taken from data of Almaty meteorological station.

Climatic data of Almaty meteorological station (2002)

Month	Perennial average		2002	
	Rainfall (mm)	Temp (°C)	Rainfall (mm)	Temp (°C)
January	35.0	-10.1	53.3	-9.5
February	38.0	-7.9	47.0	-8.3
March	70.0	-2.7	153.8	-3.1
April	100.0	10.2	177.0	10.3
May	95.0	16.4	165.7	15.5
June	56.9	21.5	101.6	22.2
July	42.8	24.1	47.0	25.1
August	30.1	20.4	24.5	22.3

2002 was a rainy year with 315 mm above the perennial average with cold and wet spring, and annual air temp of 9.3 -9.0 °C.

Laboratory experiments various herbicides were tested in pots and their effect on growth and development of field dodder was investigated in cooperation with scientists from Kazakh National al-Farabi University.

Herbicide tested in laboratory and field studies:

Commercial name	ai (%)	Active ingredient	Rate tested (kg or L/ha)
'Regio'	40	Chloridiazon + phenmedipham + desmedipham	6-8
Betanal progress	18 (6+6+6_	Phenmedipham + desmedipham + ethofumesate	4-6
Kerb	50	Pronamide	5
Pivote	10	Imazethapyr	0.8
Hurricane	48	Glyphosate-trimesium	1.0-1.5
Roundup	48	Glyphosate-isopropylamine	
Prosulfuron		Prosulfuron	
Arsenal	24	imazapyr	
Monochloracetate diethyleneglicol	95%	Monochloracetate diethyleneglicol	7-10

Results

The infected alfalfa is the main source of seeds to the seed bank in soil that spreads and infests other sensitive crops, indicating that dodder control must be focused on alfalfa. We determined that the rate of damage caused by the dodder depends on

length of parasitism. When dodder appeared on the first hay harvest, the yield declined by 35%, and on the second harvest - 31.9%. When alfalfa was infested at the end of season, the yield declined by 18.5% only as compared to non-infested control..

The amount of nutrients determining feed value of alfalfa hay when infested by dodder ('Abdygulov', 2002) reduces mineral and protein content damp ash on 3.0, damp protein-9.1, damp fat-0.85% (Table 1).

Table 1. Chemical composition of alfalfa hay infested by dodder, in percents on absolute dry weight

Treatment	(%)				Nitrogen-free extract
	Minerals	Protein	Lipids	Cellulose	
Alfalfa not infested by dodder (control)	9.7	22.1	1.6	28.1	33.5
Alfalfa infested by dodder	6.7	12.7	0.74	29.7	47.3

Following alfalfa crop, there is a large amount of viable dodder seeds in the upper soil layer. Our calculations revealed that in layer of 0-10 cm 300 seeds/m^2 are found. To reduce the potential of contamination of dodder seeds in soil we conducted fall harrowing of alfalfa infested by dodder that promoted the intensive germination of seeds. When a repeated harrowing was undertaken, dodder shoots were eliminated, and the remained dodder shoots were weak. After harrowing amount of dodder seeds reduced to 80/m^2.

Herbicide (pivote 0.8 L/ha, hurricane 1.0 and 1.5 L/ha and monochloracetate diethyleneglicol 7 and 10 L/ha) were applied in a 300 L/ha spray volume after alfalfa was first cut low and hay collected and removed. Plot size was 25 m^2 with 4 replications. The calculations were conducted in a month after the use of the herbicides (Table 2).

Table 2. The influence of the herbicides applied after first cut on dodder infestation and alfalfa yield ('Ray of the Orient', 2002)

Treatment (kg or L/ha)	Number of dodder-infested alfalfa plants/m^2			Yield (Bale/ha)
	Before treatment	After treatment	Before 2nd mowing	
Control	330.5	332.5	336.5	39.3
Pivot, 0.8	334.6	4.6	3.2	85.4
Hurricane, 1.0	343.8	8.0	4.5	77.6
Hurricane, 1.5	343.8	7.0	2.0	88.7
Monochloracetate diethylene glycol, 7.0	340.4	7.0	4.4	81.0
Monochloracetate diethylene glycol, 10.0	338.1	3.5	0.5	92.1

The data presented in Table 2 indicate that all herbicides controlled the parasite and increased the alfalfa yield. Hurricane at 1.5 L/ha and monochloracetate diethyleneglicol at 10 L/ha as well as pivot (0.8 L/ha) were the most efficient treatments. The data in Table 3 indicate that germination of dodder seeds is completely inhibited only at very high concentration of the herbicide.

Sugar beet

Sugar beet (var. Yaltushkovskaya) response to dodder and cultivation was studied in the farm 'Abdygulov'.

The following treatments were examined:

1. Hand weeding without harrowing
2. Harrowing before dodder shoot emergence
3. Harrowing after dodder shoot emergence
4. Harrowing before and after shoot emergence
5. Harrowing before and after shoot emergence complemented with Inter-row herbicide treatments (Table 4)

Table 3. The influence of herbicides on the germination of field dodder seeds in Petri dishes (10 scarified seed/dish). Laboratory experiments.

Treatment (ml/dish)	Number of germinating seeds/dish		
	Days after sowing		
	7	14	30
Control	10	10	10
Hurricane 0,01	4	4	5
Hurricane 0.02	3	4	5
Hurricane 0.05	3	4	5
Hurricane 0.1	-	-	-
Hurricane 0.5	-	-	-
Monochloracetate diethylene glycol 0.01	4	5	5
Monochloracetate diethylene glycol 0.02	3	4	4
Monochloracetate diethylene glycol 0.05	3	4	4
Monochloracetate diethylene glycol 0.1	-	-	-
Monochloracetate diethylene glycol 0.5	-	-	-
Prosulfuron 0.01	4	4	4
Prosulfuron 0.02	3	3	4
Prosulfuron 0.05	-	-	-

Table 4. Influence of cultivation and inter row herbicide treatments (Kerb 4 kg/ha) on dodder and sugar beet.

Treatment	Date applied (2002)	Dodder emergence before treatment	Dodder remaining after treatment	% control	Yield (% of hand weeding)
Hand weeding X 2	10.06 10.07	0	0	100	100
Harrowing before dodder emergence	01.06	5	2	60	74
Harrowing after emergence	14.06	8	2	75	77
Harrowing before and after dodder emergence	15.06	9	1.5	83	84
Harrowing before and after dodder emergence + Kerb	18.06 17.07	14	2	86	94

The best field dodder control was achieved when harrowing was performed before and after shoot appearance followed by inter-row treatment with the standard herbicide treatment.

Field experiment was conducted at the 'Abdygulov' farm in which several herbicides were applied post emergence on 1-2 leaf stage sugar beet. Number of dodder and sugar beet as well the beet yield and sugar content were recorded (Table 5). Plots were arranged at randomized block design (25 m^2 each) with 4 replicates.

Sugar beet was planted on 01.06.2002, and the herbicides were applied at 13.06.02. The results of the experiments show that the dodder control within the 30 days after herbicide application was 55-100% (Table 5).

Table 5. The influence of herbicides on field dodder and sugar beet productivity (Farm "Abdygulov", 2002)

Treatment (L/ha)	Field dodder		Sugar beet (plants/ m^2)	Yield (% of control)	Sugar content, %
	Plants/ m^2	% control			
No herbicides	4.5	--	4.5	100	12.5
Regio, 6.0	2	56	4	108	12.7
Regio, 8.0	2	56	4	116	12.8
Betanal progress, 4.0	1.8	61	4	114	12.7
Betanal progress, 6.0	1.3	72	4	119	12.8
Kerb 4.0	4.3	100	4.5	120	13.1

Kerb (the standard) was the most efficient treatment followed by the high rate of betanal with the same trend in yield increase. The effect on sugar content was less pronounce.

The experiment conducted in a non-agricultural land hurricane Roundup 36% were the most efficient, the control rate of dodder was between 90 and 100%. All experiments will be repeated in spring and summer of 2003 and 2004 and will be conducted in other farms as well.

C) Scientific Impact of Collaboration:

During the first year of work, we published one paper in the peer reviewed journal "Weed Research" (see above). The Kazakh partner has sent an abstract to the 15th IPPC the International Plant protection Congress scheduled for August 2003, in Beijing, China, but postponed to May 2004 due to the SARS. We hope that during the second and third year we will be able to publish a paper authored by the two groups.

D) Description of Project Impact

The project has impact on the awareness of the local scientists, extension and farmers in both countries. During the recent visit of the PI in Kazakhstan, a clear impact on major farmers was observed. In addition, the best treatments examined in the field trials were adopted by the farmers that examine them on a larger and practical scale. In spite of all difficulties described in my attached letter, which stemmed from the transition in the KIPP, the knowledge of farmers about the possibility to control dodder is improving.

E) Strengthening of Developing Country Institutions:

The visit of the PI in Kazakhstan, the participation of Dr. Zharasov in IPPC as well as the planned visit of a graduate student in the Israeli lab will strengthen the capacity of the Kazakh researchers. The difficulties in getting the proper graduate student from Kazakhstan are mostly because of changes made in the KIPP and the tendency of students who speak English to study business administration, not agronomy or biology. We hope that the suitable student will be identified and assigned to the project soon.

F) Future Work:

The work will continue as planned. In Kazakhstan, a survey outside the Almaty region will be commenced, and a taxonomic identification will be conducted. The

specimens will be characterized and photographed emphasizing their unique features for inclusion in the farmer's manual. Attempts will be made to establish the crop rotation experiments, which did not start In May 2002 due to difficulties in convincing farmers to dedicate such big plots for several years. The control measures experiments will be conducted as planned with the inclusion of the new herbicide - imazamox ('Raptor') in the program.

Section II

A) Managerial Issues:

There are no specific Managerial problem in the project apart from the fact that KIPP is undergoing a drastic process of changes that might affect the ability of the Kazakh partner, Dr. Zharasov to function properly. In addition, in both countries, the farmers are reluctant to allocate large fields for a long term (3-4 years) crop rotation experiments. This apparently stems from the general recession in the agricultural market. We will look for substations in the program that might provide similar results without being dependent on the farmers situation (small scale field experiments, etc.).

B) Budget:

There are no major changes in the budget, but there are problems in cash flow in the KIPP. During the visit to the Kazakh Institute of Plant Protection (KIPP) where Dr. Zharasov works, I met with Prof. Dr. Sagitov Abai Orazuly, and Dr, Ismuchambetov, the Director and Deputy Director of KIPP, which now belongs to the National Academic Center of Agrarian Research (NACAR), Ministry of Education & Science. Being now part of 'NACAR' increases the bureaucracy but above all, no credit is granted to scientists on Grant's money. The KIPP is willing to continue with the CDR Project and provide Dr. Zharasov with the needed accounting services. However, they can not provide him with any cash flow on the funds. That means that Dr. Zharasov has to use his own money to pay for all expenses related to the project in advance, and wait for re-imbursement from the HUJ. The HUJ pays him only for an approved Financial Report which he makes twice a year. This procedure impedes any attempt to run the planned experiments or to conduct the survey in remote places.

C) Special Concerns:

No problems.

D) Collaboration, Travel, Training and Publications:

During the first year we corresponded mostly via the electronic mail, but no travels abroad were made. Dr. Zharasov suggested sending a trainee Miss Aijan

Zhusupova, a 3rd year student at the 'Al-Faraby Kazakh National University' (biotechnology major), who speaks reasonable English and works with him on the project. I explained that according to the proposal the trainee must be a post-graduate student, M.Sc. or Ph.D. student, to make sure that she/he will use his learning to advance the knowledge in the KIPP. The search for a suitable candidate is in progress.

The Israeli group has published a paper in the **"Weed Research"** from this project (attached as part of the current report) and another paper has been published in **"Weed Science"** entitled:

Weinberg, Ts., Lalazar A. and Rubin B, (2003). Effects of bleaching herbicides on field dodder (Cuscuta campestris). Weed Science 51: 663-670. **Not included in this report. Will be sent upon request**.

This paper covers in part, the activities performed in the previous project (TA-MOU-CA 13-008).

E. Request for American Embassy Tel Aviv or A.I.D. ACTION:

None requested.