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

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Principal Investigator:

Baruch Rubin
The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, RH institute of Plant Sciences and Genetics in Agriculture, Rehovot 76100, Israel, Fax Number: ++972-8-936-2083; e-mail: rubin@agri.huji.ac.il

Co-Principal Investigator:
Prof. Shaken U. Zharasov, Kazakh Research Institute of Plant Protection
Weed Control Department, v. Rakhat, Karasai Region, Almaty Province,
Almaty, 480043, Kazakhstan, ++7- 3272-208272; e-mail: zharasov@hotmail.com

Administration Official:

Dr. E. Vardi
Director, The Authority for R&D
The Hebrew University of Jerusalem
Givat-Ram, Jerusalem 91904, Israel
Fax: 972-2-658-6638
E-mail: eran.vardi@ard.huji.ac.il

Prof. B. Sadykov, Vice General Director
Scientific Res. Institute of Plant Protection
Kazakh Scientific-Production Center of Farming and Plant Growing
Rakhat Village 483113
Karasai region, Almaty province
Kazakhstan
Fax: 8-3272-295-622
E-mail: kazniizr@nursat.kz

Grant Project Officer:
Lawrence J. Gumbiner

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C. Executive Summary

Field dodder (*Cuscuta campestris* Yonker) is a worldwide obligate parasitic weed that grows on other plants, withdrowing assimilates and water from the host plant. The parasite causes severe damage and yield losses to many crops (e.g., alfalfa, sugar beet and carrots). Dodders are amongst the worst weeds in Kazakhstan in forage and vegetable crops and may cause as much as 70-90% in yield reduction.

The principal aim of this project was to improve the management of field dodder (*Cuscuta campestris*) in major crops of Kazakhstan and provide the farmers with improved tools for identification and management of this parasitic weed. The specific aims were to better identify the species of *Cuscuta* present in Kazakhstan and Israel, their distribution and preferred host plants and to establish environmentaly-safe and cost- effective management practices to combat the parasite in major crops.

Identification and monitoring of dodder specimens in different regions of both countries was accomplished. In bothe countries, *C. Campestris* is the most damaging species detected in cultivated fields while *C. monogyna* Vahl found only on perennial shrubs and trees without significant damage. Although in Kazakhstan limited infestation of *C. lipolifolium* was also identified, this species is not important in agriculture.

Based on field and laboratory experiments, cost-effective and efficient dodder management practices were established using crop rotation combined with chemical dodder control. Investigations have shown that dodder from different countries grown alone (without host plant) or in association with various hosts, is naturally resistant to glyphosate and other herbicides that inhibit amino acid biosynthesis (AABI). These data indicated for the first time, that AABI herbicides exert their phytotoxic effect through the host plants. In spite of the complete dependence on its host for assimilated and solutes supply, the parasite maintains active enzymes such as EPSPS and ALS systems, needed for aromatic and branched amino acids biosynthesis, respectively. However, the parasite is very sensitive to herbicides that inhibit cell division.

Using oil seed rape (canola, *Brassica napus*) as a model for genetically modified crops (transgenic) that confers resistance to different herbicides, we have shown their limited capacity as means for dodder control. We proposed that Phloem-mobile herbicides such as glyphosate and other AABI inhibit assimilates translocation from the host to the parasite. AABI also change the pattern of free amino acids both in the host and parasite.

The cooperation between the two groups (KIPP and the Rehovot) has been very fruitful with a trainee (MS Zhanna Issina) visiting Rehovot laboratory twice, learning new research methods and applying them in the KIPP. Prof. Zharasov, the Co-PI from KIPP also visited Rehovot to discuss the results and summarize them for the final report. In addition, during July 2003 the PI has visited the KIPP and the experimental sites, met with the local team and the management of KIPP. Furthermore, on October 2007 a Kazakh Ph.D. student (Ms Aijan Jusupova), who was working on the project at KIPP, will visit the Rehovot Laboratory for two months, to work on field dodder as part of her Ph.D. program, and will present a paper on the work conducted in the project. During the project we developed an active and very fruitful cooperation with scientists from the USDA and Colorado State University at Fort Collins. We presented a poster at Weed Science Society of America Annual Meeting (2004) and two papers are in preparation.

We feel that most of the aims of the project were accomplished: the farmers in Kazakhstan benefit from the newly established dodder managemnt practices. But the most important acheivment is that the gain of the young Kazakh researchers that will carry the study using improved research tools.

D) Research Objectives:
The cultivated area in Kazakhstan is 36 million hectares from which no more than 3 million hectares are irrigated. In spite of the economical difficulties, 75% of the cost for plant protection are devoted to weed control. Field dodder (*Cuscuta campestris* Yuncker) is a worldwide obligate parasitic weed that causes severe damage and yield losses to many crops (e.g., alfalfa, sugar beet, safflower, onions, tomato, cucurbits and carrot). Dodders are amongst the worst weeds in Kazakhstan in forage and vegetable crops and may cause as much as 70-90% in yield reduction. Several dodder species are known to attack various crop plants. Certain weeds such *Amaranthus blitoides*, *Polygonum* spp., prosopis farcta and others are prfered host plants that serve as a bridge between crops in terms of time and space.
Based on our previous AID-funded cooperative research (TA-MOU-CA 13-008), we concluded that dodder causes severe damage to many crops both, in Kazakhstan and Israel. We also concluded that there is a need for more information on the identification and ecology of dodder species, their distribution, and in particular their response to control measures. Farmers were using herbicides without understanding their impact on the parasite, on the host plants and on the environment. Scientifically-based dodder management programs were not available.
The main objective of this study was to improve dodder managent in the major crops in Kazakhstan.
The specific aims were:

a. To determine which species of *Cuscuta* are present and damaging crop yield in Kazakhstan and Israel, their distribution, preferred host plants and to convey the information to the farmers,.
b. To establish cost-effective and environmentally-sound dodder management practices based on crop rotation and judicious use of herbicides.

The Problem addressed: *Cuscuta campestris* Yuncker (field dodder) is a nonspecific above-ground holoparasite, and as such is totally dependent on its host plant for assimilates nutrients and water supply. *C. campestris* is widely distributed with wide range of host species. There are more than 170 described *Cuscuta* species in the genera that are obligate parasites. They parasitize various plants in the world (Parker and Riches, 1993), of which 19 are known in Kazakhstan (Arstangaliev and Ramasanov, 1977) and 12 in Israel (Fienbrun-Dothan and Danin, 1991). Most of the 170 species are found primarily in the Americas from Canada to Argentina (Hickman, 1993). Dodders also parasite trees, shrubs and other herbaceous plants.
The hosts include crop and weeds - dicots, some monocots but never grasses (Parker and Riches, 1993). Effective control is extremely difficult to achieve as dodder seeds remain viable in the soil for a long time, and continue to germinate and emerge throughout the warm seasons. In addition, the parasite and host association is so strong that it is difficult to control the parasite without damaging the host plant. Amino acid biosynthesis inhibitors (AABI) are one of the herbicide groups reported to have an effect on *C. campestris* growth.

The genus *Cuscuta* belongs to the Convolvolaceae family and comprises over 100 different species. All species are absolute above-ground parasites and develop as yellow-orange, rootless and leafless long twining stems. *Cuscuta* species have functioning chloroplasts (Hibberd et al., 1998) and contain a small amount of chlorophyll and other accessory pigments (Dinelli et al., 1993; Weinberg et al., 2003) and probably are more self sufficient then expected regarding their ability to synthesize proteins (Holm et al., 1997). In order to finish a life cycle the parasite must make contact with a host.
In this genus *C. campestris* (syn. *C. pentagona*) is usually considered the most damaging species probably due to its wide geographical distribution and large range of hosts (Parker and

Riches, 1993; Dawson et al., 1994; Holm et al., 1997). *C. campestris* is widely distributed in warm and temperate regions but can proliferate in extreme hot and cold conditions (Holm et al., 1997). *C. campestris* is usually annual but in certain conditions especially during a mild Israeli winter (but not that prevailing in Kazakhstan) it may survive the winter and resume growth during the next spring. *C. campestris* is considered a serious weed in lucerne, tomato, pepper, potato, carrot, onion, sugar beet, tobacco, alfalfa, clover and other legumes causing severe damage and crop losses (Holm et al., 1997). *Cuscuta* seeds are believed to be spread around the world with unclean crop seed and once a field is contaminated these will continue germinating and producing new seeds over a long period of time. *Cuscuta* seeds remain viable in the soil for a long time and unlike root parasites do not require a specific stimulant for germination (Dawson, 1994). Mechanical or chemical scarification of the seed coat is sufficient to break dormancy after which *Cuscuta* seeds will germinate independently of the presence of a host plant (Dawson et al., 1987). After germination the seedling develops towards the light in search for a host and on contact it coils around the host stem. Thigmotropic responses and chemical recognition will cause the parasite to penetrate the host tissue and develop pre-haustoria and haustoria within a few days (Tsivion, 1979; Press et al., 1990; Vaughn, 2003). Once the haustorium is established within the host its epidermal cells will develop into 'searching hyphae', elongate within the host tissue, and meet with the vascular bundles of the host.

Assimilate movement between the host and parasite was thought to be apoplastic (Wolswinkel, 1986; Jescke et al., 1994) however, the movement of green fluorescent protein (GFP) from a transgenic host to *Cuscuta* (Haupt et al., 2001) indicates the existence of a symplastic pathway between the host and parasite. Recent microscopic examinations of the parasite's haustoria revealed a unique plasmodesmata structure (Vaughn, 2003) and it is suggested that at the site of attachment the parasite is not identified by the host as an invader but rather cooperates with it to build chimeric cell walls between them (Vaughn, 2002). Once the haustorium of the parasite is well established within the host, this highly efficient absorption system allows the parasite to divert resources - water, amino acids and assimilates - from the host into the parasite (Tsivion, 1979; Dorr, 1987). Wolswinkel (1984) describes a remarkable parallel between assimilate transport from the host phloem to the *Cuscuta* haustoria and transport from maternal tissue to the embryo in developing pods of a legume. Furthermore, it was shown that when *Cuscuta* and a developing pod compete for assimilates, the sink activity of the parasite is much stronger while the sink activity of the fruit can disappear completely. The mechanism of enhanced assimilate unloading from the host phloem into the parasite haustorium is yet unknown. However, the unloading was shown to be under metabolic control, since its effectiveness was lost at 0°C or in the presence of metabolic inhibitors (Wolswinkel et al., 1984). Wolswinkel (1984) attributes the competitive power of the *Cuscuta* sink to the strategic localization of the haustorium adjacent to the sieve tubes in the main vascular bundles of the host plant.

***C. campestris* control**: Effective control of *C. campestris* is extremely difficult to achieve as parasite seeds remain viable in the soil for a long time, and continue to germinate and emerge throughout the warm seasons. Prevention would probably be the most effective and most economical method for *Cuscuta* control (Dawson, 1987; Parker and Riches, 1993). Selective chemical control of C*uscuta* if difficult and there are two major concerns that arise from the nature of attachment and association between the parasite and its host. The first concern is that although all the biological mechanisms exist in the parasite, many of them are not essential for its survival. This means many herbicides such as photosynthesis inhibitors will have no effect on the *Cuscuta*. The second is the fact that the xylem and phloem elements of the host and parasite are connected, therefore only a highly selective herbicide that will not

harm the host can be used. Fer (1984) suggested that due to the low transpiration rate of the parasite, herbicides that are translocated in the host's xylem should not be used for *Cuscuta* control. It was argued that such xylem-mobile compounds move and accumulate mostly in the host's organs (with higher transpiration rates) and may damage the host more than the parasite (Fer, 1984). On the other hand a phloem-mobile herbicides such as glyphosate, and other AABI applied to the host plant may accumulate selectively in the parasite because of it being a stronger sink and inhibit the parasite growth without harming the host (Liu and Fer, 1990; Bewick et al., 1991; Liu et al., 1991; Dawson et al., 1994; Nir et al., 1996; Nadler-Hassar et al., 2002).

It was shown that glyphosate (Fer, 1984; Liu & Fer, 1990; Bewick et al., 1991) and imazaquin (Liu et al., 1991) applied to the host foliage accumulated in the apical part of *Cuscuta* in concentrations which are much higher than those found in the apical bud and young leaves of the treated host. Unfortunately, in most cases, host crops were damaged by these treatments (Parker & Riches, 1993; Nir et al, 1996) and the parasite was not always adequately controlled (Dawson et al., 1994). Newly introduced transgenic crops resistant to AABI are reported to be efficient in the selective control of *Orobanche* spp. (Joel et al., 1995) and *Striga* spp. (Kanampiu et al., 2001) and remain to be tested as a mean to control *Cuscuta.*

The enzyme 5-enolpyruvylshikimate-3-phospate synthase (EPSPS) is considered the primary site of action for glyphosate. It is a key enzyme in the shikimate pathway and biosynthesis of aromatic amino acids (phenylalanine, tyrosine and tryptophan) and is responsible for condensing shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP) into EPSP. Glyphosate acts as a competitive inhibitor of PEP and once it binds to the EPSPS-S3P complex the connection is practically irreversible (Devine et al., 1993). Inhibition of the pathway in treated plants leads to overproduction and accumulation of shikimate (Amerhein et al., 1980) and it has been reported that in some cases treated sink tissue will contain shikimate and shikimate-3-phosphate up to 16 percent of their dry weight (Schulz et al., 1990). The exact cause for plant death is not yet understood, but there is evidence that glyphosate reduces carbon fixation and starch production in source leaves and decreases the ability of sink leaves to draw assimilates (Geiger & Bestman, 1990; Geiger et al., 1999). A large portion of secondary metabolites in plants derive from the shikimic pathway and it has been suggested that uncontrolled flow of carbons to the pathway and the lack of essential amino acids could disrupt the formation of secondary metabolites such as lignin, flavonoids, chlorophyll and phytohormones. (Devine et al., 1993). It has been reported that supplying IAA to tobacco callus and 2,6-dihydroxyacetophenone (a phenolic compound that effectively inhibits IAA metabolism) to *Teucrium canadense L.* can alleviate some of the glyphosate effects (Lee & Starratt, 1989). Alleviation and reversal of glyphosate phytotoxic effects by the addition of exogenous aromatic amino acids was not always achieved especially in higher plants (Jaworski, 1972; Gresshoff, 1979; Devine et al., 1993) but these results support the hypothesis that besides the EPSPS, glyphosate has additional secondary site(s) of action (Lee and Starratt, 1989).

As expected, not all plants are similarly affected by glyphosate and the level of tolerance to the herbicide varies between plants. Up to date an altered EPSPS target site was discovered only in a naturally occurring glyphosate-resistant *Eleusine indica* (goosegrass) biotype (Baerson et al. 2002) and it has been suggested that such mutations are scarce since they would lead to reduced survival of the weed. The tolerance of several members of the Convolvulaceae family to glyphosate is achieved by a combination of several mechanisms such as high EPSPS activity induced by the herbicide and greater shikimate pathway activity. This high activity provides the plant with higher amino acid pools that help protect the plant from EPSPS inhibition (Westwood & Weller 1997). The resistance of *Dicliptera chinensis* is attributed to an increase in the levels of the EPSPS mRNA and protein and may be

posttranscriptionaly regulated (Yuan 2002). As a rule, glyphosate is stable in plants since most of them are unable to metabolize and degrade the herbicide (Devine et al. 1993), legumes are probably an exception. Their tolerance to low glyphosate rates could be attributed their ability to degrade the herbicide (Lee 1980). Unlike most higher plants, legumes can metabolize some of the glyphosate absorbed by the plant (Nandula et al. 1999) thus increasing the tolerance of the plant.

Unlike Glyphosate which is practically the only herbicide that inhibits the EPSPS, there are five groups of herbicides that inhibit acetolactate synthase (ALS) activity: sulfonylureas (SU), imidazolinones (IMI), triazolopyrimidines (TP), pyrimidinylthiobenzoates (PTB) and sulfonylaminocarbonyltriazolininones (SCT) (Tranel & Wright, 2002). ALS is a key enzyme in the biosynthesis of branched chain amino acids. The condensation by ALS of two pyruvate molecules will form one 2-acetolactate, a precursor of leucine and valine and the condensation of one pyruvate and one 2-ketobutyrate will form 2-acetohydroxybutyrate, a precursor of isoleucine (Devine et al., 1993). As with glyphosate, besides the direct effect of ALS inhibitors on the target enzyme it has been shown that these herbicides cause a decrease in assimilate export out of the treated leaves and import by source leaves, suggesting that one of the outcomes of ALS inhibition would be, **'starvation'** due to lack of assimilate supply (Bestman et al., 1990; Hall and Devine, 1993; Kim and Vanden Born, 1996). This may explain the fact that after herbicide application young developing meristems are the first tissue to show signs of stress. Alleviation of the growth inhibition caused by ALS inhibitors to higher plants was achieved with the addition of the appropriate amino acids to the growth media (Ray, 1984), indicating that external supply of the missing amino acid (like in host-parasite association when the host plant supplies amino acid and other solutes to the parasite) should prevent the damage. Based on these data we developed our work hypothesis:

As an absolute parasite, *C. campestris* being totally dependent on assimilates supply from the host plants, operates a strong sink, consuming all there needs in terms of amino acids from the host. Hence, inhibiting the production of amino acids in the parasite should not cause any growth inhibition in the parasite. The fact that AABI such as glyphosate, SU and IMI herbicides severely injure the parasite indicates the involvement of additional mechanism such as "**starvation**". We hypothesize that the parasite dies due to inhibited translocation of assimilates from the host, whereas lack of amino acids is a minor factor.

This hypothesis was examined in cooperation with Dr. Dale Shane (USDA, ARS, Fort Collins, CO), Dr. P. Westra and Dr. S. Nissen (Colorado State University, Biological Science and Pest Management, Weed Science Lab, Fort Collins, CO) using US dodder populations. In order to support our hypothesis and based on the mode of action of the AABI, we recorded the effect of these herbicides on the free amino acids pool (content and proportions), in the parasite and the host plant.

E. Methods and Results:

The Kazakh Report

Introduction

Field dodder (*Cuscuta campestris* Yuncker) is a worldwide obligate shoot parasite plant, that causes damages and yield losses to a wide range of host species. World flora According to the Russian Institute of Plant Quarantine the field dodder can be found in 165 countries worldwide. There are 19 species of dodder from three families infesting of plants in

Kazakhstan. The dodders are divided to two major types: Thin shoots (0.2-1 mm) that infest crops and weeds and thick shoots (2-4 mm) that parasitize on perennial shrubs and trees.

Dodder specimens on the biological and morphological particularities divided for 3 subgenus: *Grammica, Cuscuta, Monogyna*. The main kinds of dodders are *Cuscuta campestris* Yuncher – Gzammica, *Cuscuta monogyna – Cuscuta monogyna* Vahl) and *Cuscuta lupuliformis* Kroc Ker).

After germination the seedling if the parasite elongates in a search for a host and once a suitable host is found the parasite will use its haustoria to penetrate into the host tissue and make contact with the vascular bundles of the host. Wide distribution of field dodder makes it one of most harmful parasite in the world. The main regions for dodder spreading, growing and harmfulness are irrigated zones of Kazakhstan (Almaty, Dzambyl, Southern and Eastern regions).

Field dodder may reduce crop productivity (sugar beet, onion and carrot) by 70-80% and seeds of alfalfa almost by 80-95% (Zharasov, 1999, Rubin, 1996). Our research project was located in areas where harmfulness and damage of dodders are visible in the territory of Kazakhstan.

Tasks of the studies on the first years (2002-2005):

1. Monitor the infestation and define the identity, structure and number of dodders in crops in the Eastern and Southern of Kazakhstan, the regions atre: Zhambyl, Almaty and Akmola.
2. Examine the response of field dodder to agro-technical measures.
3. Examine the response of field dodder to new herbicides in alfalfa, sugar beet, carrot and safflower in field and laboratory.
4. Investigate the role and efficacy of herbicides in the field dodder control on non-agricultural lands.

Methods and Results

Field experiments were conducted in the following farms "Ray of the Orient", "Abdygulov", "Kairat", "Adal" in Talgar and Ili districts in Almaty region. The soil in Talgar district farms is middle-loamy light chestnut, 1.9-21% organic matter (OM), pH – 8,0. The soil of the Ili district farms is light grey, 1-1.7 OM.

Climatic Conditions:

Weather analysis for 2002-2005 was taken from data collected by Almaty meteorological stations. 2002 was rainy year with 516 mm above the perennial average with cold and wet spring, and average annual air temp. of 19ºC. In 2003 the spring also was cold and long. Total rainfall during the vegetation period was 785mm. Average monthly temp was 14.2ºC as compared o the perennial norm - 18.4ºC.

In 2004, total rainfall was 413mm that was higher than the norm by 173mm, average monthly temp was 20.4ºC as compared to the norm of 18.4ºC. In 2005, from April till September the rainfall was 330mm, Average monthly temp was similar to the perennial average daily temperature of 19.4-20.8ºC.

Specimen collection:

During 2003-2005, 207 herbarium specimens were collected from Almaty, Zhambyl, Akmola regions, South and East of Kazakhstan. The results are presented in **Appendix I**. It is clear from the data presented that the dominant dodder species in Kazakhstan is *Cuscuta*

campestris Yuncker (subgenus Grammica) parasitizing on safflower, alfalfa, sugar beet, carrot, onion, melon and various weeds.

Several populations of *C. monogyna*, *C. australis*, *C. lupiliformis* were also found. Herbarium material was collected in all farms of Kazakhstan regions, identified, photographed and documented. We cooperated with Dr. Kudabaeva G.M., Associated Professor, a leading scientist from the Laboratory of High Plants Flora of Botany and Phytointroduction Institute.

Field dodder has wide distribution on the territory of Kazakhstan. North of Kazakhstan – Astana region 222 ha; Kostanai - 12 ha, Karaganda – 480 ha, Pavlodar – 62 ha, West of Kazakhstan – 2 612 ha, Aktobe region - 100 ha; Atyrau – 554 ha; Kyzylorda – 25 800 ha, Mangystau region – 11 ha; East of Kazakhstan - 5200 ha; South of Kazakhstan – 27 388 ha, Zhambyl - 52 392 ha; Almaty region – 499378 ha, Almaty – 286 ha. All area 165 040 ha (Map 1).

Map 1. Dodder distribution throughout Kazakhstan (field dodder = ■)

Table1. Climatic data measured at the Almaty region meteorological stations (2002-2005).

Year	**Rainfall (mm)**						**Temperature (^{0}C)**						Rainfall (mm)	Annual (Ave.)
	IV	**V**	**VI**	**VII**	**VIII**	**IX**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**		
2002 - Almaty station	177	165.7	101.6	47	24.5	27.0	10.3	15.5	22.2	25.1	22.3		542.8	19.8
	100	95	56.9	42.8	30.1	26.0	10.2	16.4	21.5	24.1	20.4		350.8	18.5
2003 - Station Talgar district	184.6	187.8	139.1	246.5	13.5	13.9	0.4	10.8	17.2	18.5	21.5	16.3	785.4	14.2
	56.5	61.6	53.9	26.6	21.2	13.5	10.4	16.4	21.2	24.1	22.1	16.5	233.3	18.4
2004 - Station Kaskelen district	105.6	88.1	73.3	84.9	36.1	24.7	10.2	19.3	24.8	23.4	22.8	17.1	412.7	20.4
	60.8	61.0	53.9	26.6	21.2	15.9	10.4	16.4	21.2	25.1	22.1	16.0	239.4	18.5
2005- Almaty station	54.9	93.2	69.3	14.1	64.7	33.9	14.6	19.3	21.7	24.3	20.8	24.3	330.1	20. 8
	56.5	61.6	53.9	26.6	21.2	34.3	10.4	16.4	21.2	24.1	22.1	22.3	254.1	19.4

Our observations have shown that the soils in "Adal". "Ray of the Orient". "Kisanova". "Abdygulova" farms are heavily infected with field dodder. We suggested to include the following crops in the crop[rotation: Sudangrass (*Sorghum vulgare* var *sudanense)*, winter wheat and maize which are not infected by field dodder and offered the following crop rotation..

Table 2. The recommended crop rotation.

Years	**Crop rotation I**	**Crop rotation II**	**Crop rotation III**
2006	Sudangrass	Winter wheat	Winter wheat + alfalfa
2007	Grain corn	Soybean	Alfalfa
2008	Soybean	Safflower	Alfalfa
2009	Winter wheat	Winter wheat	Winter wheat
2010	Sugar beet	Sugar beet	Safflower

When onion, potato, sunflower, tomato and other crops would be cultivated, they will be included in the rotation according to these recommendations.

Dodder-infected alfalfa is the main source of dodder seeds to the seed bank in the soil. This reservoir serves as the inoculum that spreads and infests other sensitive crops, indicating that dodder control must be focused on alfalfa.

We observed that the rate of damage caused by the dodder depends on length of parasitism in alfalfa. When dodder appears on the first hay harvest, the yield declined by 35%, and on the second harvest – 32%. When alfalfa was infected at the end of season, the yield declined only by 18.5% as compared to non-infected control.

The amount of nutrients in the hay determines the feed value of alfalfa hay: Dodder infested alfalfa contains reduced mineral and protein content damp ash on 3.0. damp protein – 9.1. damp fat – 0.85% (Table 2).

Table 3**. Effect of dodder infestation on the chemical composition of alfalfa hay (% of total dry weight).**

Treatment	**Minerals**	**Protein**	**Lipids**	**Cellulose**	**Nitrogen-free extract**
	% of total dry weight				
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

There is a large amount of viable dodder seeds in the upper soil layer. In layer of 1-10 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. The amount of dodder seeds after harrowing was reduced to 80 seeds/m^2.

In 2002-2004 we conducted an experiment in the "Ray of Orient" farm and examined several herbicides for dodder control in alfalfa. Herbicides (Pivote 0.8 l/ha; hurricane 1.0 and 1.5 l/ha and monochloracetate diethyleneglycol 7 and 10 l/ha) were applied in a 300 l/h spray volume after alfalfa was first cut low and hay collected and removed. Plot size was 25 m^2 with 4 replications. The data presented in Table 2 indicated that herbicide controlled the parasite and increased the alfalfa yield.

The data presented in Table 3 indicate that all herbicides controlled the parasite and increase the alfalfa yield. The best treatment were Hurricane (1.5 l/ha) and Monochloracetate diethylene glycol (10 l/ha) and Pivote (0.8 l/ha).

Field dodder injuries in oil- and vegetable- crops in the irrigated area of Kazakhstan are described in Figs. 1, 2, and 3.

Table 4. The influence of the herbicides applied on dodder-infested alfalfa on the hay yield ("Ray of the Orient" and Kyzyl tu 3. 2002-2004).

	Number of dodder infested alfalfa plants/m^2			
	Before treatment	**After treatment**	**Before 2nd mowing**	**Yield (bale/ha)**
Treatment (L/ha)	**"Kyzyl tu 3" 2002-2004**			
Control	340.2	343.8	347.5	48.5
Pivot. **0.8**	343.5	5.2	2.9	95.0
Hurricane **1.0**	346.6	5.5	3.2	90.5
Hurricane **1.5**	347.3	5.3	1.5	95.5
	"Ray of Orient" 2002			
Control	330.5	332.5	336.5	39.3
Pivot. **0.8**	334.6	4.6	3.2	85.4
Monochloracetate diethyleneglycol. **7**	340.4	7.0	4.4	81.0
Monochloracetate diethylene glycol **10**	330.1	3.5	0.5	92.1

Fig. 1. Field dodder parasitizing sugar beet

Fig. 2. Field dodder parasitizing safflower.

Fig. 3. Field dodder parasitizing onion.

Sugar beet (var. Yaltushevskaya one-seeded) response to dodder and other weeds was studied in the farm "Abdygulov". The following treatment 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 emergence complemented with inter-row herbicide treatment

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 (**Table 5**).

Field dodder control experiment was conducted with several herbicides that were applied post emergence at the 1-2 leaf stage sugar beet. Sugar beet was planted on 01.06.2002 and the herbicides were applied at 13.06.02. Number of dodder and sugar beet as well the beet yield and sugar content were recorded. Plots were arranged at randomized block design (25 m^2 each) with 4 replicates. The results of the experiment show that dodder control within the 30 days after herbicide application (DAT) was 55-100% (Table 6).

Table 5. **Influence of cultivation and inter row herbicide treatments on dodder infestation and sugar beet yield.**

Harrowing	**Date applied**	**Dodder emergence before treatment**	**Dodder remaining after treatment**	**Control (% of untreated)**	**Yield (t/ha)**
None (hand weeding)	10.06/ 10.07	0	0	100	0.31
Before dodder emergence	01.06	5	2	60	0.23
After dodder emergence	14.06	8	2	75	0.24
Before + after dodder emergence	01.06+1 5.06	9	1.5	83	0.26
Inter row herbicide treatment	18.06/ 17.07	14	2	86	0.29

Figure 4. Herbicide treatment in a commercial field.

Table 6. Effect of herbicides on field dodder and sugar beet productivity (Farm Abdygulov, 2002). Evaluation times were: 1= before treatment; 2= 30DAT; 3=60DAT.

Traetment (L/ha)	Evaluation time	Field dodder		Sugar beet (Plants /m^2)	Yield (t/ha)	Sugar content (%)
		Plants/ m^2	% Control			
Control	1	3		4	25.0	12.5
	2	4		4		
	3	4.5		4.5		
Regio **6**	1	4		3.8	27.0	12.7
	2	1	75	4		
	3	2	56	4		
Regio **8**	1	4		4	29.0	12.8
	2	1	75	4		
	3	2	56	4		
Betanal Progress **4**	1	5		5	28.6	12.7
	2	1.5	70	4		
	3	1.8	61	4		
Betanal Progress **6**	1	4		4	29.8	12.8
	2	0.8	81	4		
	3	1.3	72	4		
Kerb **4** (standard)	1	4.3		4.5	30.0	13.1
	2	0	100	4.0		
	3	0	100	4.3		

Kerb 50, the standard herbicide, was the most efficient treatment followed by the high rate of betanal progress, with a yield increase in the same trend. The sugar content in the herbicide-treated plots was higher (by 0.300.6%) than that of the untreated control.

In 2003 we continued testing herbicides efficacy and safety in sugar beet. Sugar beet was planted on May 20 in a commercial field in Baiserke Agro. We tested several preemergence applications of Frontier (90% dimetanamid) at 1.2 and 1.7 L/ha, postemergence treatments at 12 leaves stage with Nortron (42% ethofumesate) at 2 L/ha and Kerb-50 (50% propyzamide or pronamide) at 5 kg/ha. The treatments were applied on 25 m^2 plots replicated 4 times.

The results are given in **Table 7**. Kerb was the best treatment being highly effective in reducing dodder infestation by 100%. The high soil moisture due to rainfall guarantied high efficacy of this herbicide which resulted in increasing root yield by 11.0 t/ha and sugar content by 1.2 % above the control.

Fig. 5. Pre-emergence herbicide treatment.

Frontier applied preemergence at low rates (1.2-1.7 L/ha) reduced dodder infestation by 70-80% and improved the roots yield by 4.0-6.0 t/ha and sugar content by 0.3-0.5%. Nortron applied at 2 L/ha reduced dodder infestation by 60%, and slightly improved root yield 5.0 t/ha and sugar content by 0.4%.

In 2005 year our field experiments on the carrot, sugar beet and safflower were continued in various locations. Experiments were conducted using preemergence herbicides (Piramin turbo. 52% ai – 5 L/ha. Frontier optima 72% ai – 1.7 L/ha) to control field dodder in sugar beet (var. Yaltushevskaya). Plot size was 25 m^2 with 4 replications.

The postemergence herbicides (Betanal Progress, 18% ai.– 4 L/ha; Bicept Garant, 27% ai – 3 L/ha, and a combination of both herbicides - Betanal Progress 3 L/ha and Lontrel – 0.3 L/ha) were applied in the 2-3 true leaves stage of sugar beet and the dodder growth was 3-4 cm on a background of pre-emergence applied herbicides . Pre-emergence herbicides were applied after sowing sugar beet on 20 April and post-emergence herbicides were applied on 18 May at the 2-3 leaves stage.

The results have shown (Table 8) that the combination of pre- and post-emergence herbicides (Frontier Optima, Betanal Progress and Lontrel) were the best treatment for effective control of field dodder and increasing yield by 4.6 T/ha.

In 2003-2004 we examined the effect of several herbicides applied pre-emergence (Stomp. 4-6 L/ha. Racr 2-3 L/ha) and early post-emergence in carrot planted at the end of May (27.05.03 and 29.05.04). Stomp (pendimethalin) and Racer (flurochloridone) were applied pre-emergence and Gesagard (prometryn) – standard was applied early post-emergence at the 1 to 2 leaves stage. Plot size was 25 m^2 with 4 replications.

Table 7 – Effect of herbicides on field dodder and sugar beet yield (Baiserke Agro, 2003). Evaluation times were: 1= before treatment; 2= 30DAT; 3=60DAT.

Treatment (Kg or L/ha)	Evaluation time	Field dodder infestation		**Yield (t/ha)**	**Sugar content (%)**
		shoot/m^2	**Mortality (%)**		
Control	1 2 3	**4** 5 5		20.0	12.0
Kerb **5.0**	1 2 3	**4.5** 0 0	100 100	31.0	13.2
Frontier **1.2**	1 2 3	**2.0** 1.5 1.5	50 70 70	24.0	12.3
Frontier **1.7**	1 2 3	**1.5** 1 1	63 80 80	26.0	12.5
Nortron **2.0**	1 2 3	**4.5** 1.5 2.0	67 60	25.0	12.4
LSD_{095}				4.5	

Table 8. Influence of the combination of pre-emergence followed by (***fb***) a post-emergence treatment on field dodder, the control of other weeds and sugar beet yield (Kairat. Talgar district, Almaty region, 2005). Evaluation times were: 1= before treatment; 2= 30DAT; 3=60DAT.

Treatment (L/ha)	**Evaluat-ion time**	**Dodder**		**Other weeds**		**Yield (t/ha)**	**Sugar (%)**
		Shoot/m^2	**Control (%)**	**Shoots/ m^2**	**Control (%)**		
Control	1 2 3	6 7 11		15 25 30		26.0	13.4
Piramin Turbo **5** ***fb*** Betanal Progress **4**	1 2 3	2 2.0 1	60 71 91	3 3 2	80 88 93	29.5	13.5
Frontier Optima **1.7** ***fb*** Betanal Progress **4**	1 2 3	3 2 1.5	40 71 86	4 3 2	73 88 93	29.5	13.5
Piramin Turbo **4** ***fb*** Bicept garant **3**	1 2 3	2 1.5 0.5	67 79 95	3 2 0	80 92 100	30.1	13.6
Frontier Optima **1.7** ***fb*** Bicept garant **3**	1 2 3	3 2 1	50 72 91	4 2 0	73 92 100	29.7	13.5
Frontier Optima **1.7** ***fb*** Betanal Progress **3** +Lontrel 0.3	1 2 3	3 1 0.5	50 86 95.4	4 1 1	84 96 97	30.6	13.5
LSD_{095}						3.2	

Fig. 6. Post-emergence herbicide treatment in sugar beet.

Stomp at 6 L/ha and Racer at 3 L/ha effectively reduced field dodder infestation by 75% and before harvest by 80% and were effective also in general weed control. These herbicides increased carrot yield by 3.2 to 3.5 t/ha.

In 2005 (Kyzyl tu 2 and Baiserke Agro) we examined in the effect of combination of pre-emergence herbicide Stomp (4 L/ha) three days after carrot sowing (28.05.05) followed by post-emergence application of Gesagard at 3 L/ha at the 2 leaves stage (08.06.05) when the seedlings were 2-3 cm high. The results are shown in Table 10. The combination of sequential herbicide application improved the control of dodder and other weed and increased yield by 4.0 t/ha as compared to untreated control.

Table 9. Influence of herbicide treatment on field dodder, other weeds and carrot yield (Kyzyl Tu 1, Almaty Region 2003 -2004). Evaluation times were: 1= before treatment; 2= 30 DAT; 3= 60 DAT.

Treatment (L/ha)	**Evaluation time**	**Dodder**		**Other weeds**		**Yield (t/ha)**
		Shoot/ m^2	**Control (%)**	**Shoots/ m^2**	**Control (%)**	
Control	1	5		26		13.9
	2	5		32		
	3	6		37		
Gesagard **2**	1	5		25		14.5
	2	2	60	4	84	
	3	2	60	5	87	
Gesagard **3**	1	4		27		16.1
	2	1	75	5	83	
	3	2	60	2	95	
Stomp **4**	1	1	72	3	88	15.4
	2	1	75	4	87	
	3	2	57	4	89	
Stomp **6**	1	1	75	3	88	16.4
	2	1	80	3	90	
	3	1	80	2	94	
Racer **2**	1	1	72	5	80	14.8
	2	2	40	5	83	
	3	2	68	4	89	
Racer **3**	1	1	75	4	84	15.9
	2	1	80	4	87	
	3	1	80	3	91	
LVD_{095}						1.3

Table 10. Influence of different concentration of herbicides on field dodder and carrot yield (Almaty region. Kyzyl tu district. Baiserke farm – 2005). Evaluation times were: 1= before treatment; 2= 30 DAT; 3= 60 DAT.

Treatment (L/ha)	Evaluation time	Field dodder		Other weed		Yield (t/ha)
		Shoots/ m^2	% Mortality	Shoots/ m^2	% Mortality	
Control	1	8		20		12.0
	2	11		35		
	3	16		40		
Stomp. 4 **fb** Gezagard 3	1	5	37.5	3	85.0	16.0
	2	1	90.9	3	91.4	
	3	1	93.7	5	87.5	

In 2004 we conducted field dodder control in safflower (var. Malutinskii. farm Adal. Ili district. Almaty Region). Field dodder and other annual weeds were treated by pre-emergence herbicides Trifluralin (Nitran 30% ai) at 4 and 6 L/ha; Pendimetalin (Stomp 33% ai) at 4 and 6 L/ha; Dimethanamid (Frontier, 90% ai) at 1 and 1.2 L/ha and post-emergence with Bentazon (Bazagran 48% ai) and Bentazon + Acilfluorfen (Galaxitop 36+12% ai).

As shown in Fig. 2, it is visible that under the conditions of the Southeast of Kazakhstan, field dodder is very harmful weed for safflower. When attached to safflower, dodder exhausts the host from its nutrients and water and consequently weakens and inhibits the host's growth and biomass development. The seeds of field dodder have matured 18-20 days earlier than the safflower and they intensively disseminated before the crop is harvested. The effects of the tested herbicides on field dodder and productivity of safflower are presented in Table 10.

Herbicides were applied pre-emergence immediately after safflower sowing on April 28. 2004. Field dodder began to parasitize on the safflower and weeds on May 10. The herbicides did not cause any negative influence on growth and development of safflower. The post-emergence herbicides, Bazagran and Galaksitop were applied at the 2-3 true leaves stage of safflower on a background of soil applied herbicides.

The results clearly indicate that all herbicides resulted in a significant yield increase due to dodder and other weeds control. The most effective herbicide was dimethanamid (frontier) at both rates that increased safflower yield by 0.5 t/ha as compared to the untreated control. Trifluralin applied at 4 and 6 L/ha reduced dodder infestation for the first 30 days by over 80%, later on its efficacy was gradually declined, reaching 56 to 65% at the harvest time. Pendimethalin was quite similar to trifluralin in dodder control, but was less effective in controlling other weeds.

We also studied the effect of pre-emergence treatment of Nitran + Frontier followed by different post-emergence herbicide treatments on dodder control (Table 11). Although all post-emergence treatments controlled field dodder and significantly increased yield over the control. Galaksitop applied at 2.0 L/ha was the best treatment. controlling field dodder and increasing yield by 0.55 T/ha. The contribution of the post treatment is important to extend the affect made by the pre-emergence treatments which fade up in time.

Table 10. Influence of pre-emergence herbicides (applied at 28/4/04) on the control of field dodder and other weed and safflower yield (2004 year).

Treatment (L/ha)	Evaluation date	Weed control (%)		Yield (t/ha)	Yield increase (t/ha)
		Dodder	Other weeds		
Control	10.05	58	70	0.82	
	10.06	61	76		
	20.07	59	74		
Nitran **4.0**	10.05	81	90	1.06	0.24
	10.06	70	71		
	20.07	56	67		
Nitran **6.0**	10.05	87	92	1.22	0.4
	10.06	78	74		
	20.07	65	70		
Stomp **4.0**	10.05	85	88	1.05	0.25
	10.06	62	65		
	20.07	50	54		
Stomp **6.0**	10.05	87	90	1.20	0.38
	10.06	65	72		
	20.07	60	72		
Frontier **1.0**	10.05	79	85	1.30	0.48
	10.06	70	78		
	20.07	62	68		
Frontier **1.2**	10.05	83	90	1.34	0.52
	10.06	75	81		
	20.07	65	71		

This data demonstrate that a combination of pre and post-emergence herbicide treatment can reduce field dodder infestation in safflower and significantly contribute to the yield.

In 2005, experiments with combination of pre and post-emergence herbicides were continued. The post-emergence herbicides (Bazagran, 2 L/ha and Galaksitop, 1.5 L/ha) were applied in the 2-3 true leaves of safflower (25th of May) on a background of pre-emergence herbicides (Stomp, 6 L/ha and Frontier, 2 L/ha). Phenological observation of the growth and development of safflower were conducted during the vegetative period. The results shown in Table 13 indicate that the combination of pre and post emergence herbicides reduced the dodder infestation for the first 30 days by 67-75%, at 60 days by 85-90% and by 93.3-96.7% at the harvest time. The most effective among tested herbicide combination Stomp, Frontier

and Galaksitop at rates of 1.5 L/ha. Crop yield was increased by 0.4-0.42 t/ha as compared to untreated control.

Table 11. Effect of herbicides applied post-emergence on dodder control and safflower yield. All plots were pre-treated with nitran + frontier (PK Adal. Ili district. Almaty region, 2004). Evaluation times were: 1= before treatment; 2= 30 DAT; 3= 60 DAT.

Treatments	**Evaluation time**	**Dodder control (%)**	**Plant height (cm)**	**Weight of 1000 seeds (g)**	**Yield (t/ha)**	**Yield increase (t/ha)**
Control	1 2 3	60 63 59	90.4	32.6	0.84	___
Bazagran **1.5**	1 2 3	70 80 82	88.8	36.4	1.2	0.36
Bazagran **2.0**	1 2 3	 85 86	86.5	34.5	1.29	0.45
Galaksitop **1.5**	1 2 3	 87 89	90.3	37.6	1.3	0.46
Galaksitop **2.0**	1 2 3	 88 92	90.0	38.2	1.39	0.55

In 2006 we started experiments with several herbicide treatments in 2-3 leave stage of sugar beet (the 25th of June): 1. Control; 2. Kerb 50 (6 kg/ha) (standard); 3. Betanal Progress (4 L/ha); 4. Betanal progress 3 L/ha+Lontrel 300 (0.3 L/ha); 5. Betanal progress (3 L/ha) + Kerb 50 (3 kg/ha). First evaluation (08.07.06) showed that most of field dodder shoots were dried. This experiment will be continued.

Table 13 – Influence of herbicides applied pre-emergence followed by (fb) a post-emergence treatment on field dodder and safflower (PK «Adal». 2005). Evaluation times were: 1= before treatment; 2= 30 DAT; 3= 60 DAT.

Treatment (L/ha)	Evaluation time	Field dodder		Other weeds		Yield (t/ha)
		shoots/ m^2	% Mortality	shoots/ m^2	% Mortality	
Control	1	8		30		0.85
	2	10		45		
	3	15		40		
Stomp **6 fb** Bazagran **2**	1	2	75	5	83.3	1.21
	2	1	90	7	84.4	
	3	2	87	4	90.0	
Stomp **6 fb** Galaksitop **2**	1	2	75	4	86.7	1.27
	2	1	90	6	86.7	
	3	0.5	97	2	95.0	
Fronter **2 fb** Bazagran **2**	1	2.5	69	5	83.3	1.2
	2	1.5	85	6	86.7	
	3	1	93	3	92.5	
Fronter **2 fb** Galaksitop **1.5**	1	2	75	5	83.3	1.25
	2	1	90	5	88.9	
	3	1	93	1	97.5	

Laboratory studies:

The effect of herbicides on germination of field dodder seeds was examined under laboratory conditions (Table 14). The effect of commercial formulation of prometryne (Gezagard). trifluralin (Treflan). prosulfuron (Prosulfuron) and glyphosate (Hurricane) on dodder germination and early growth was examined at rates 0.01; 0.02; 0.1; 0.2 and 0.5 ml in square Petri dishes (9.0 cm diameter). Acid scarified seeds were dispersed on a filter paper soaked with the herbicide solution. Number of germinating seedlings and shoot length was recorded 7, 14 and 30 days after sowing.

Data was shown that Hurricane strongly inhibited seed germination and shoot growth even when applied at low concentrations. High concentration of Prosulfuron (0.05 ml) also provided good effect. Gezagard and Treflan did not influence much the germination process but strongly inhibited dodder shoot growth. In 2006 in laboratory conditions was examined herbicide Goal 4E (oxyfluorfen) at 5 rates (0.01 ml. 0.02 ml. 0.05 ml. 0.1 ml. 0.2 ml). The results have shown that at 0.05 ml and 0.1 ml field dodder did not germinate. In addition we tested 5 experimental herbicides synthesized by the Institute of Chemistry of Academy of sciences of the Republic of Kazakhstan, but these herbicides did not inhibit the growth and development of field dodder.

Table 14. Effect of commercial herbicide formulations applied in Petri dishes on the germination and development of field dodder seedlings.

	Germination (seedlings/dish)			**Shoot length (cm)**		
	Time of measurement (days after sowing)					
Treatment (ml/dish)	7	14	30	7	14	30
Control	10	10	10	5	8	14
Gezagard. 0.02	8	9	9	2.9	3.8	4.5
Gezagard. 0.05	7	9	9	9.6	2.7	3.6
Gezagard. 0.1	7	8	8	0.9	1.8	2.9
Gezagard. 0.2	6	8	8	0.5	0.9	2.5
Gezagard. 0.5	6	7	7	0.5	0.6	1.8
Treflan. 0.02	7	9	9	2.8	3.7	4.6
Treflan. 0.05	7	9	9	2.3	3.7	3.9
Treflan. 0.1	6	9	9	1.2	1.5	2.8
Treflan. 0.2	6	8	8	1.2	1.5	2.7
Treflan. 0.5	5	8	8	0.5	1.4	2.6
Hurricane 0.01	2	2	2	0.2	0.3	0.3
Hurricane 0.02	2	2	2	0.2	0.2	0.3
Hurricane 0.05	0	0	0	0	0	0
Hurricane 0.1	-	-	-	-	-	-
Hurricane 0.2	0	0	0	-	-	-
Prosulfuron 0.01	4	4	4	0.5	1	2
Prosulfuron 0.02	3	3	3	0.5	1	1

Laboratory method that was developed in FAR (sand bioassay), was employed under the Kazakh laboratory conditions. Different concentration of herbicides were examined in the *Cuscuta campestris* and its hosts sugar beet and safflower. We examined the following herbicides: Betanal Progress (phenmedifam + desmedifam + ethofumesate). Bazagran, Harmony, Frontier and, Piramin turbo (chloridazon or pyrazon). The results are shown in the following figures:

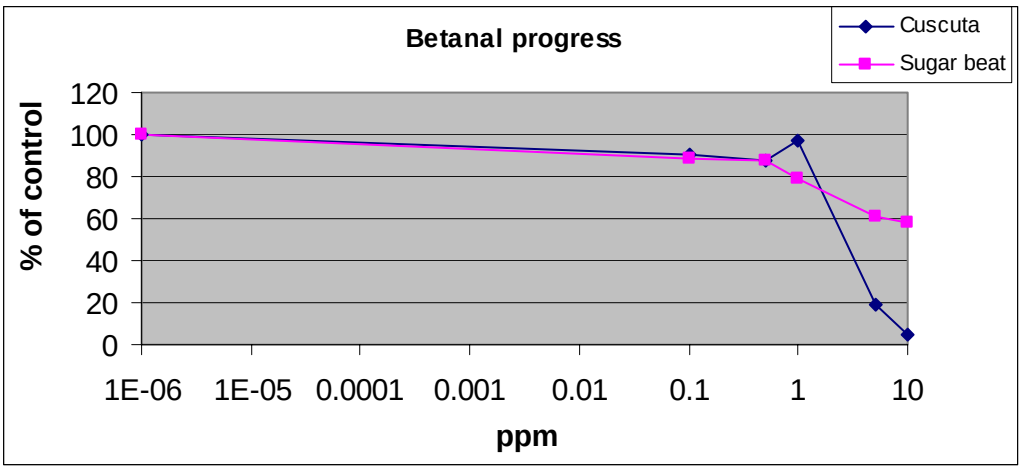


Fig. 7. Effect of Betanal Progress on the elongation of *Cuscuta campestris* shoots and sugar beet roots.

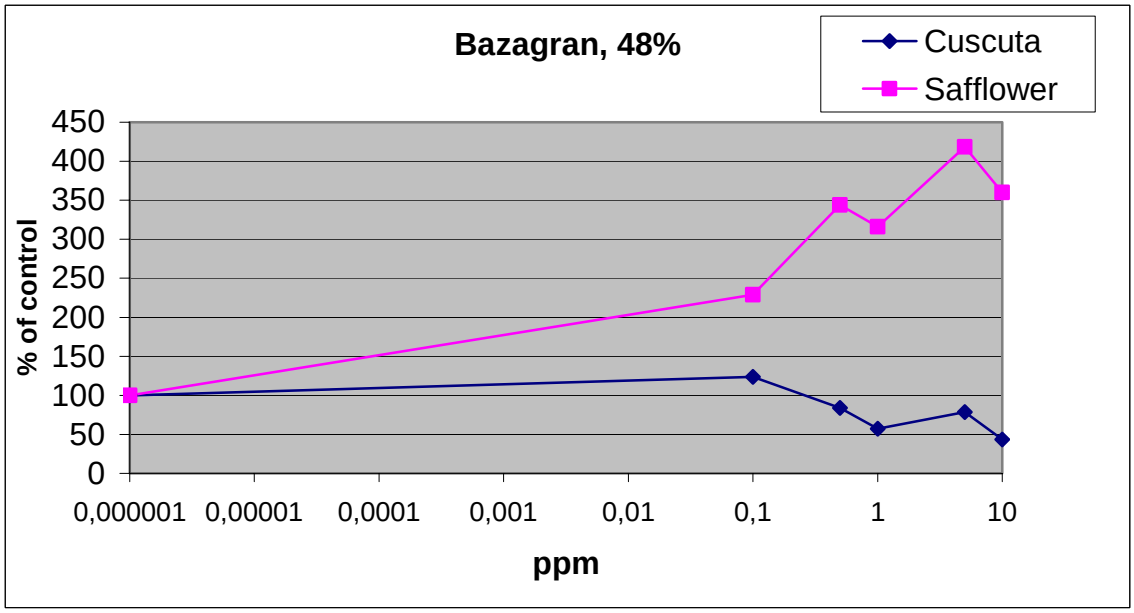


Fig. 8. Effect of Bazagran on the elongation of *Cuscuta campestris* shoots and safflower roots

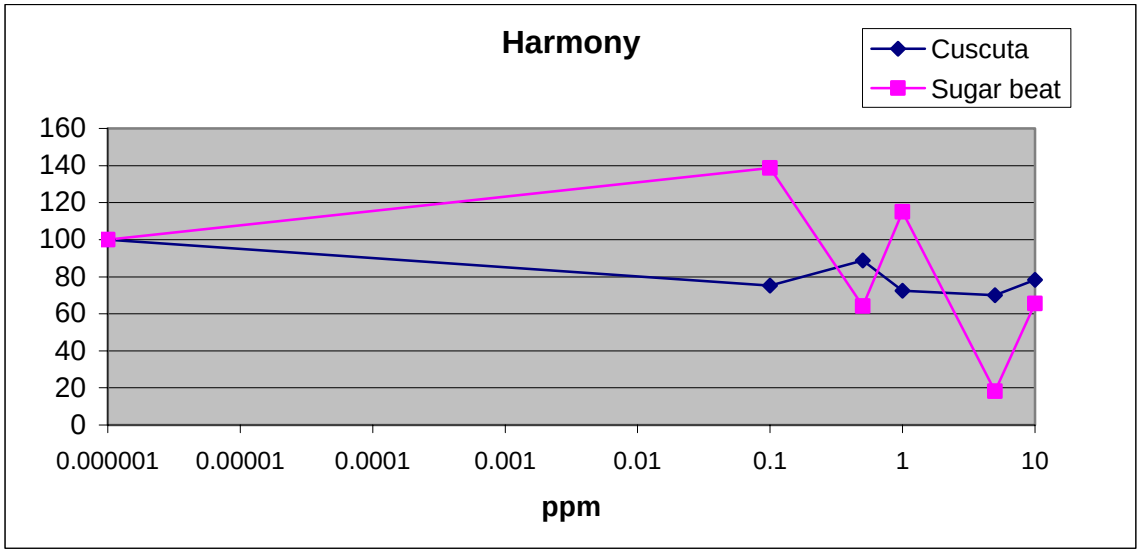


Fig 9. - Effect of Harmonyi on the elongation of *Cuscuta campestris* shoots and sugar beet roots

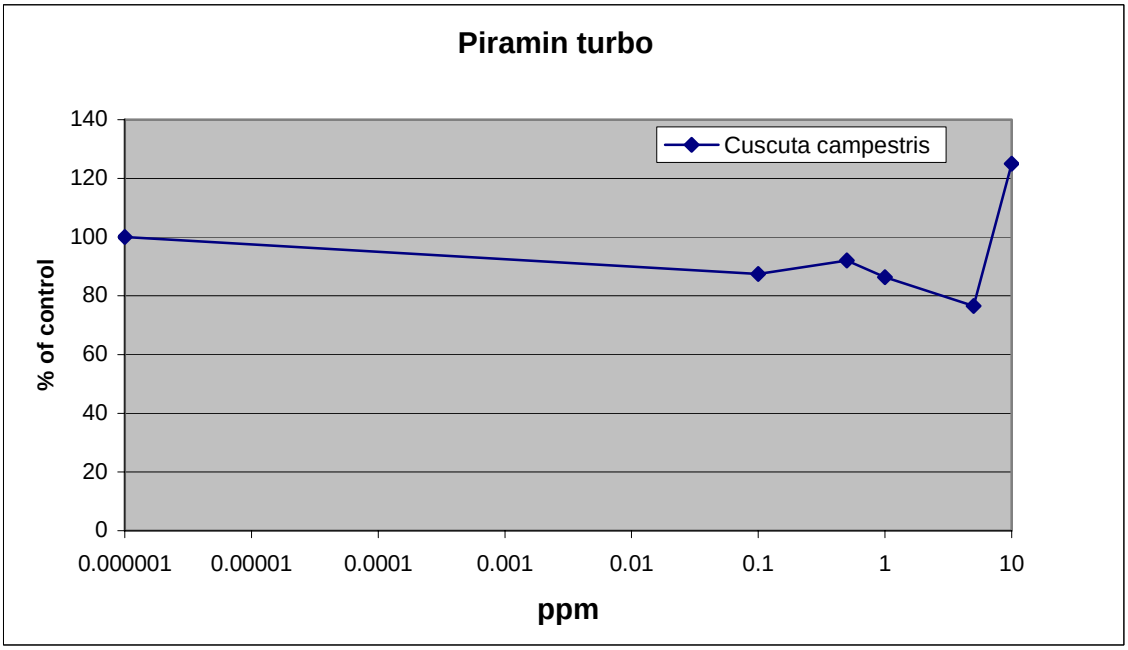


Fig 10. Effect of piramin turbo on the elongation of *Cuscuta campestris* shoots

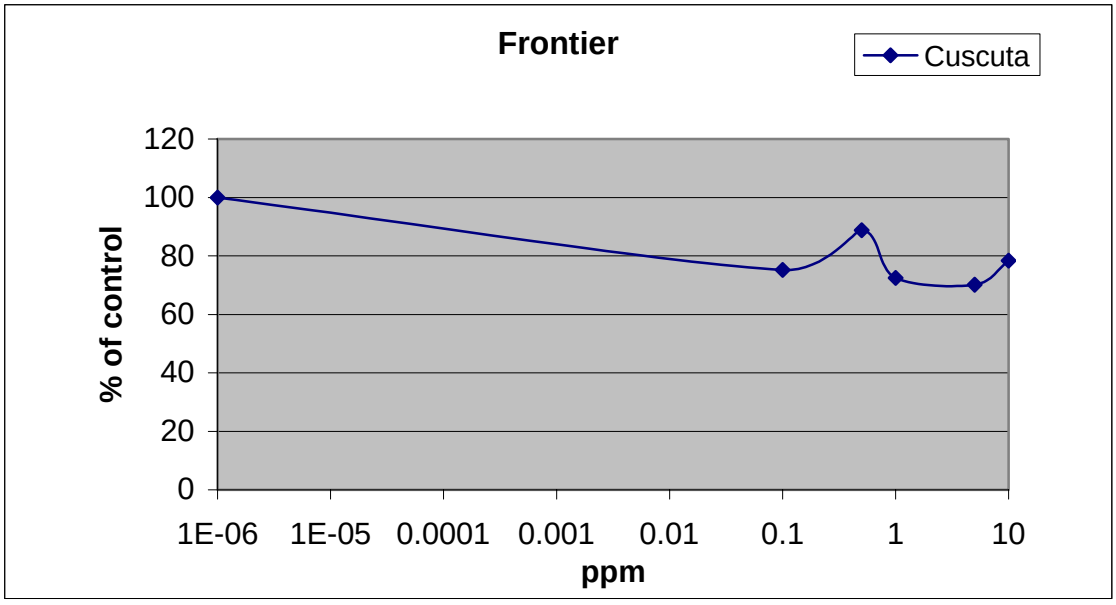


Fig. 11. Effect of Frontier on the elongation of *Cuscuta campestris* shoots.

In 2006 we conducted pot experiments in order to test the response of different crops (sugar beet, safflower, alfalfa) to field dodder. The results have showed that *C. campestris* reduced crop shoot weight by 71, 67, and 70% respectively (Fig. 12 and Table).

Fig 12. *Cuscuta campestris* developed on sugar beet .

Table 15. Response of sugar beet, safflower and alfalfa to field dodder

Host	Plant biomass grown without dodder (g)	Plant biomass with dodder (g)			Biomass reduction due to dodder (%)
		Total	Host alone	Dodder alone	
Sugar beet	2.85	1.06	0.83	0.23	71
Safflower	1.93	0.88	0.63	0.25	67
Alfalfa	0.91	0.51	0.27	0.14	70.

Field dodder had controlled on non-agricultural lands during 3 years. We used several formulations of Glyphosate: Hurricane at 3 L/ha and Tornado 4 L/ha. These treatments provided similar and excellent dodder control - 100%.

Conclusions

Field dodder is the most damaging parasite and has wide distribution on the territory of Kazakhstan. Field dodder causes damage to different crops: alfalfa, sugar beet, safflower, onion, carrot and other crops. The critical period of the dodder damage for alfalfa, sugar beet, and onion starts on 2-3 leaves stage, when dodder stems (6-7 cm) with haustoria are attached to the plant. Chemical analyses showed. that the amount of nutrients determining feed value of alfalfa hay when infested by dodder reduced mineral and protein content: ash by 3.0%, protein – 9.1%, and fat by 0.85%. Herbicides application (Pivote 0.8 l/ha, Hurricane 1.0 l/ha) increased the alfalfa yield by 4.6 to 5.1 t/ha and reduced the field dodder infestation by 98 to 99.6%. The best field dodder control was achieved when harrowing was performed before and after the dodder shoot emergence followed by inter-row treatment. This combination significantly increased sugar beet yield by 6.0 t/h.

The contribution of pre- and post-emergence herbicides: Frontier Optima, Betanal Progress and Lontrel, were the best treatments in controlling field dodder. This treatments increased yield by 4.6 t/ha. These herbicides reduce the field dodder infestation by 91-95%. The most effective herbicide was Kerb 50 (applied at high spray volume of 600 L/ha) and reduce field dodder by 100% with an increase in yield of 11.0 t/ha and sugar content by 1.2% over that of the control.

On carrot, we examined effect of combination of pre-emergence (Stomp 6 L/ha. Racer 3 L/ha) and post-emergence applied prometryne (Gezagard) that reduced dodder damage by 94% and increased yield by 4.0 t/ha.

The most effective treatment on safflower among the tested herbicide combinations were Stomp, Frontier and Galaksitop at rates of 1.5 L/ha. Crop yield was increased by 0.4-0.42 t/ha as compared to untreated control and reduced field dodder infestation by 93-97%.

Field dodder was effectively controlled on non-agricultural lands during 3 years. The best treatment was Glyphosate (Hurricane; Tornado) at 3 and 4 L/ha. These treatments provided excellent dodder control. In laboratory conditions Hurricane 0.01 ml/dish, and Prosulfuron 0.01 ml/dish reduced seed germination and shoot growth.

The Israeli Report:

Cuscuta identification

More than 50 dodder population were collected throughout the country from cultivated and non-cultivated habitats and identified. The samples were parasitizing carrot, tomato (Figs. 1 and 2), chickpea (Fig. 3), watermelon (Fig. 4), ornamental plants (Fig. 5), prostrate pigweed (*Amaranthus blitoides*) (Fig. 6 and 7), *Polygonum bellardii*, *P. arenastrum*, camelthorn (*Alhagi graecorum*) and Syrian mesquite (*Prosopis farcta*) (Fig. 8).

Although 12 *Cuscuta* spp. are described in the Israeli flora (Feinbrun-Dothan and Danin (1991), 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* (Fig. 9), which parasitized perennials (shrubs, bushes and trees) in the northern part of the country.

Fig. 1. *Cuscuta campestris* infesting processing tomatoes.

Fig. 2. *Cuscuta campestris* infesting processing tomatoes. Note the yellow spots on other rows.

Fig. 3. *Cuscuta campestris* infesting chickpeas. Left – Early infestation; Rioght Late infestation. Note the heavy damage caused to the crop (right).

Fig. 4. *Cuscuta campestris* infesting watermelon.

Fig. 5. *Cuscuta campestris* infesting lilac chastertree (*Vitex* spp.) an ornamental plant.

Fig. 6. *Cuscuta campestris* parasitizing prostrate pigweed (*Amaranthus blitoides*). Note the amount of flowers and seeds developed, that will enrich the soil seed-bank with fresh seeds.

Fig. 7. *Cuscuta campestris* parasitizing prostrate pigweed (*Amaranthus blitoides*). Note that the crop (Sunflower) plants are not infested.

Fig. 8. *Cuscuta campestris* on Syrian mesquite (*Prosopis farcta*).

The facts that field dodder may parasitize crops and weeds and the longevity of its seeds in the seed bank, reduce the value of crop rotation as an instrument in the combat against the

parasite. Poor weed control in a non-host crop such as sunflower (Fig. 7) allows the parasite to complete the life cycle with seed production on weeds such prostrate pigweed and enrich the seed bank, abolishing the value of crop rotation. The described above situation is a common situation in Israel as well as in Kazakhstan, emphasizing the importance of the need for rational herbicide use and understanding the behavior of herbicide in the host-parasite association.

Fig. 9. *Cuscuta monogyna* parasitizing the shrub *Ziziphus spina-christi*.

In the proposal we suggested "to record during the sample collection, any presence of natural enemies such as pathogens and insects, specifically *Smicronyx* spp. for future use". Unfortunately, the weevile *Smicronyx* spp. (COLEOPTERA: CURCULIONIDAE) was not detected in any of the fields surveyed in Israel. However, a sporadic distribution of dodder plants inoculated with *Smicronyx* spp. were found in several sugar beet fields in Kazakhstan in (Fig. 10), but no reduction in dodder infestation was observed. Future studies should be directed to estimating the real damage caused by the weevil to the parasite, in terms of biomass and seed production, reduced damage to the crop, etc.

Fig. 10. *Cuscuta campestris* inoculated with *Smicronyx* (left). Note the galls formed by the insect on the parasite hyphea (Arrows). Right: *Smicronyx* inoculated C. campestris grown on sugar beet and Venice mallow (*Hibiscus trionum*).

Response of *C. campestris* to AABI herbicides

When herbicides are applied on a "holoparasite" such as *C. campestris* is grown in association with host plants (the "normal" way), it is very difficult to determine whether the observed damage caused to the parasite was direct or indirect (via inhibition of the host plant). We overcame these difficulties by using two different approaches:

1. We examined the effect of AABI herbicide on dodder seedlings ("seedling bioassay") during the first 5 days when they grow without being attached to host plant.
2. We examined the response of the parasite when grown in association with an altered target site herbicide-resistant host plant.

In the course of our studies we developed a new technique – "seedling bioassay" (Fig. 11) using sand in Petri dishes as described in details in our paper published in: *Weed Research (2003) 43: 341-347* (see **Appendix II**).

Using the "seed bioassay" we discovered (and reported) for the first time that *Cuscuta* seedlings grown without host, are highly resistant to all AABI herbicides tested. This includes glyphosate, sulfonylureas and imidazolinones. The I_{50} value (herbicide concentration that inhibits dodder growth by 50%) for glyphosate was 8 folds 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 (Table 1 and Fig. 11).

Fig. 11. A bioassay for testing the response of plants to herbicides Seedling bioassay." Note the dodder shoot growth as compared to sorghum and cotton root growth.

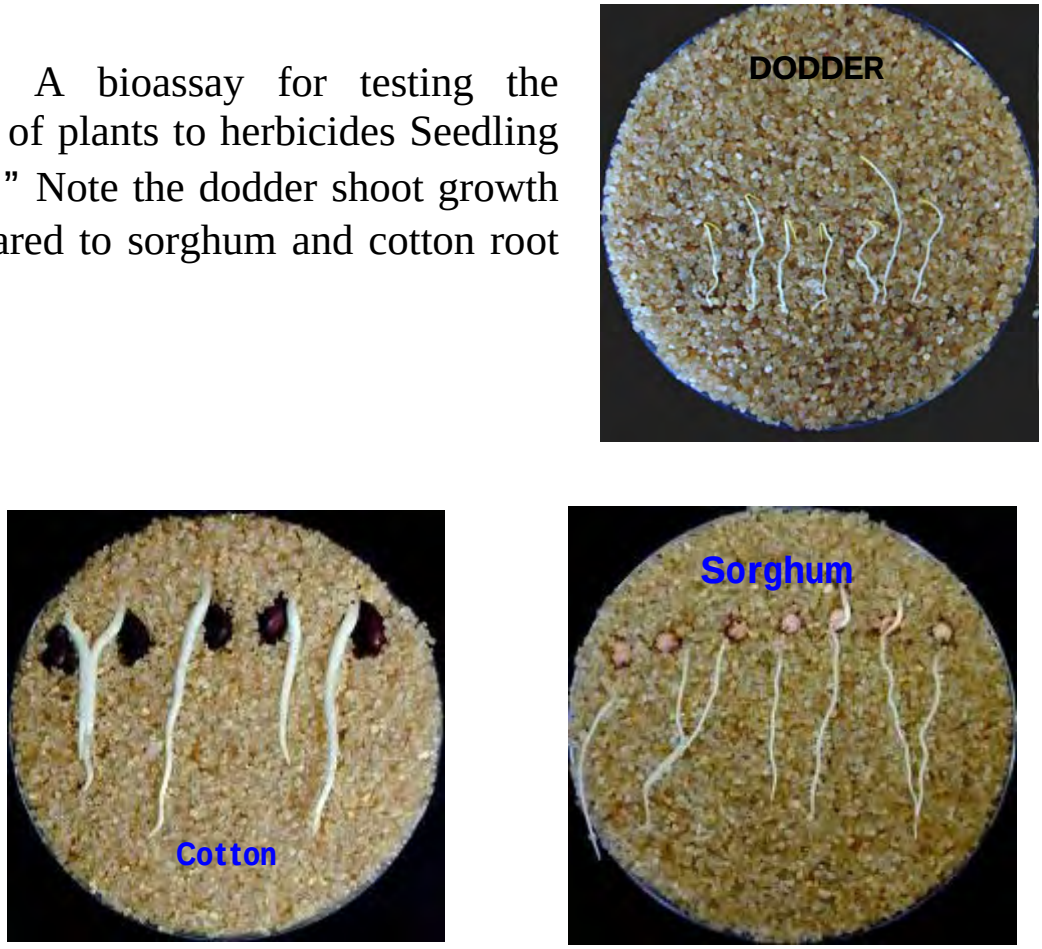


Table 1: Response of *C. campestris* and other plants to herbicides applied in the sand filled Petri dish bioassay. The log-logistic model (Seefeldt *et al.*, 1995) was used to calculate the I_{50} values from the dose-response curves of shoot (*C. campestris*) or root (other plants) elongation.

			RR		SuR		SuR-	SuS-
	Dodder	**Sorghum**	**Cotton**	**Wheat**	**Tomato**	**Maize**	***A. blitoides***	***A. blitoides***
Herbicide					I_{50}			
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	-	-	1000	-	-	-
Pyrithiobac (µM)	140	<0.001	-	-	250	-	-	-
Trifluralin (µM)	1	1	-	-	-	-	-	-

With the help of our US collaboration, we have shown that this unique resistance to AABI may exist not only in field dodder but also in other *Cuscuta* species (Table 2). Seeds of three different dodder species: *C. campestris*, *C. gronovii* and *C. subinclusa* were collected in different parts of the US, were grown and propagated on oilseed rape in the green house to maintain a homogenous supply of viable seeds before testing.

Table 2. Seedlings response to AABI herbicides as determined in the germination paper assay. The I_{50} values were calculated using a log-logistic model. GRR=transgenic glyphosate resistant oil seed rape; IR= imidazolinone resistant oil seed rape.

	Herbicide I_{50} (mM)	
Plant species	**Glyphosate**	**Imazamox**
*Cuscuta campestris**	24.0	9.18
*C. Gronovii**	13.1	2.61
*C. subinclusa**	21.1	8.92
*Sorghum***	0.08	0.001
GRR- oilseed rape **	25.6	-
IR oilseed rape **	-	1.13

*Shoot elongation was measured; **Root elongation was measured;

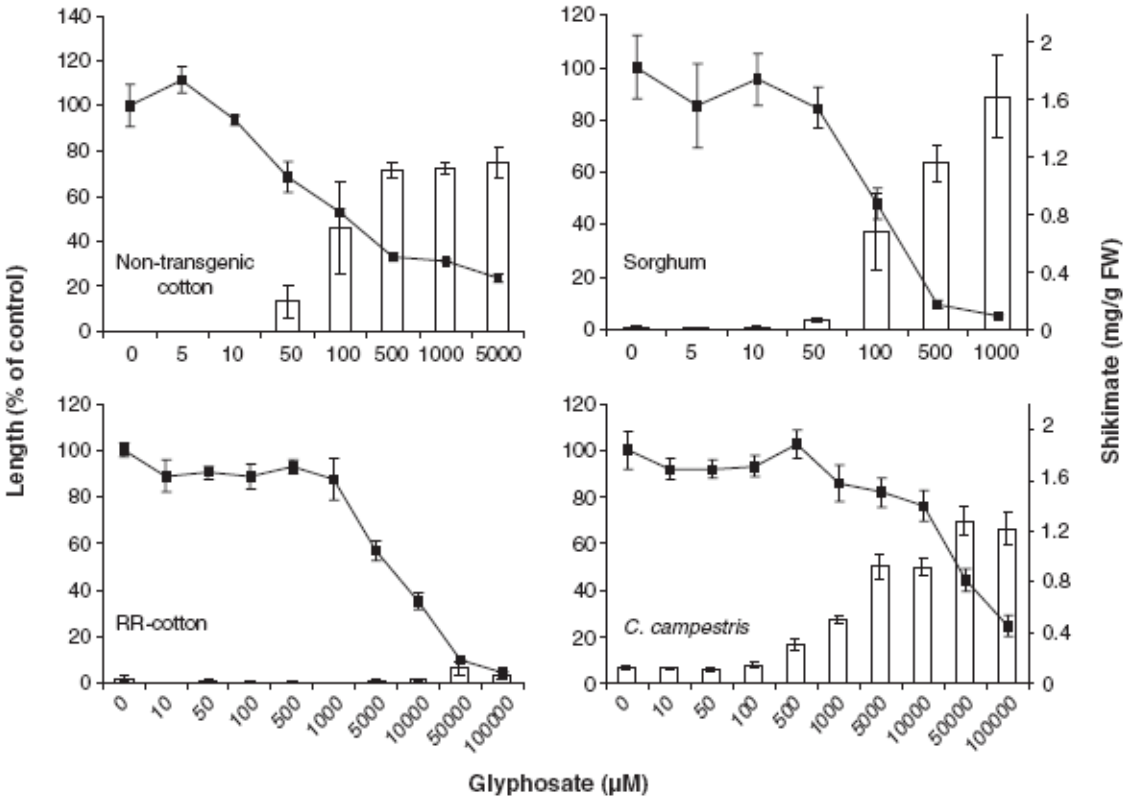


Fig. 12. Shikimic acid accumulation (**bar**) and root (cotton, sorghum) or shoot (*C. campestris*) length (**line**) at different glyphosate concentrations (µM). Vertical bars represent the standard errors of the means. Vertical bars represent the standard error of the means.

Glyphosate interrupts with the biosynthesis of aromatic amino acids (phenylalanine, tyrosine and tryptophan) in plants by inhibiting the 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 and Bestman 1990). The uncontrolled flow of carbons to the shikimic pathway and the lack of essential amino acids disrupts additional pathways (Devine et al. 1993). The exact cause of the plant's death after glyphosate application is not yet clear but there is evidence that besides inhibiting EPSPS, glyphosate may also inhibit translocation of assimilates from source leaves to various sinks (Geiger and Bestman 1990; Geiger et al. 1999).

The data in Fig. 12 indicate that *Cuscuta* seedlings responded to the glyphosate treatment by accumulating shikimate, but their shoot length was affected only when exposed to more that 10.000 µM of the herbicide, whereas the root length of the sensitive plants such as cotton and sorghum was dramatically inhibited at 100 µM of glyphosate. Possible explanation will be detailed later in this document, but these data support our working hypothesis that starvation might be the indirect effect of AABI on dodder when it grown in association with host. These data were published in Planta (*Nadler-Hassar, T., Goldshmidt, A., B. Rubin and S. Wolf (2004). Glyphosate inhibits the translocation of GFP and sucrose from a transgenic tobacco host to Cuscuta campestris Yunk. Planta 219: 790-796*) **see Appendix II**.

Earlier studies established that glyphosate is accumulates mainly in the parasite after application to a *C. campestris*-parasitized host plant (with no tolerance to glyphosate) due to the parasite's strong sink activity. As a result, the parasite growth is inhibited with no significant damage to the host (Fer 1984; Liu and Fer 1990; Nir et al. 1996). We have shown

that application of glyphosate to a parasitized glyphosate-resistant (RR) sugar beet as host, results in an initial injury to the parasite, but at a later stage the parasite was able to recover and resumed normal growth (Nadler-Hassar and Rubin 2003). Collectively, these results indicate that the parasite has some natural mechanism of tolerance to glyphosate. However, when the parasite-host plant are treated with AABI together, the parasite is initially injured due to lack of amino acid supply, but later on the parasite should resume growth and draw the needed supply of amino acids from the host. Hence, inhibition of amino acid biosynthesis does not have to be critical for the parasite's survival, and cannot be the sole mechanism by which glyphosate affects *C. campestris* development.

Our hypothesis is that *C. campestris* is injured by AABI herbicide mainly due to the inhibition of assimilates and solutes transport from the host leading to "starvation" of the parasite rather than the direct herbicide's effect on amino acid biosynthesis.

In the present study we developed a unique experimental system by which we were able to directly characterize the kinetic of proteins and sucrose transport from a host plant to *C. campestris*. Tobacco plants expressing a green fluorescent protein (GFP) in the sieve element-companion cells (SE-CC) complex, under the control of the *Arabidopsis thaliana* sucrose transporter (AtSUC2) promoter (Oparka et al. 1999), were employed to monitor the effect of glyphosate on the traffic of the fluorescent protein to the parasite. The influence of glyphosate on phloem transport of carbon was based on pulse-chase experiments after [^{14}C]–sucrose application to source leaves. We describe here the major results only. Details on the methods used and the results are given in the manuscript in **Appendix II**).

Glyphosate (36 µg a.e.) was applied to the third leaf (10 1-µl drops) of tobacco plants. *C. campestris* and host plant tissue were sampled for confocal laser scanning microscopy (CLSM, model LSM510, ZEISS, Jena, Germany) examination, shikimic acid determination, and protein extractions for immune blots 3, 7, 10 and 14 days after glyphosate treatment (DAGT). Assimilates movement from the tobacco host to the parasite was followed using [^{14}C]sucrose, autoradiography, and liquid scintillation counting. Shikimate content was determined according to Singh and Shaner (1998) with modifications of Cromartie and Polge (2000).

In glyphosate <u>non-treated</u> the early florescent signals (GFP) were detectable in the *C. campestris* apex 14 DAS, and reached its highest level 25 DAS (Fig 13), followed by a steady decline that paralleled the decline in the vitality of the host. The immune blots with specific GFP antibody supported the observations made by CLSM. In glyphosate-treated plants the parasite growth and development ceased 3 DAGT followed by partial necrotic shoots, while other shoots turned green and began to develop new, compact and deformed shoots from which, at a later stage, a new developing apex emerged.

The data in Fig. 13 show that 3 DAGT, there was a significant reduction in GFP levels in the parasite as compared to the increasing GFP levels seen in *C. campestris* growing on untreated hosts. At 7 DAGT, the GFP in *C. campestris* growing on herbicide-treated hosts had reached undetectable levels. At the same time the GFP level in *C. campestris* growing on untreated hosts was slightly lower than that detected 25 DAS but remained significantly higher than that detected in *C. campestris* growing on herbicide-treated hosts. The changes in [^{14}C]sucrose accumulation correlated with those seen in GFP accumulation. Forty-eight hours after glyphosate application, the [^{14}C]sucrose in *C. campestris* was reduced by 50% as compared to that in *C. campestris* attached to untreated hosts (Fig. 14).

Shikimic acid accumulation was used as an indication of EPSPS inhibition by glyphosate. Shikimate was detectable in the parasite as early as 1 DAGT, confirming that the herbicide had rapidly reached its target site. Shikimate accumulated continuously over time in the

parasite, but not in the host. Three DAGT, the apex of non-parasitized hosts had accumulated high shikimate levels; on the other hand, very little shikimate was accumulated in the apex of glyphosate-treated hosts parasitized with *C. campestri*s (data not shown).

Based on these observations, we propose that the parallel reduction in GFP and [^{14}C]sucrose accumulation in *C. campestris* during these 3 days is not a result of host damage and reduced source activity but a weakening effect of glyphosate on the parasite's ability to act as a sink and withdraw assimilates from the host. The high shikimate levels detected in the parasite 1 DAGT confirmed the uptake of glyphosate by the parasite and its arrival to an active EPSPS target site there, indicating also the high strength of the sink employed by the parasite. The reduction in [^{14}C]sucrose and GFP accumulation following glyphosate application supports our hypothesis that the parasite growth is inhibited, at least in part by starvation for assimilates rather than by direct herbicide inhibition of the parasite's EPSPS. Although glyphosate inhibits the EPSPS of *C. campestris* attached to a resistant host or in this case to an undamaged host, the parasite has an alternative source from which to withdraw the needed amino acids. The parasite should not therefore be affected by the herbicide, as the latter normally exerts its effect via the inhibition of amino acid biosynthesis, which can be alleviated by amino acid replenishment from exogenous sources (Jaworsky 1972) or from the host. The inhibition of sucrose and GFP accumulation in *C. campestris* could be explained by the host's reduced ability to export assimilates or by a loss in the parasite's sink strength (Geiger and Bestman 1990). The low shikimate levels found in glyphosate-treated host leaves and the lack of visible injury to the host suggest that the reduction in GFP and sucrose accumulation in the parasite 3 DAGT is a result of reduced parasite sink strength.

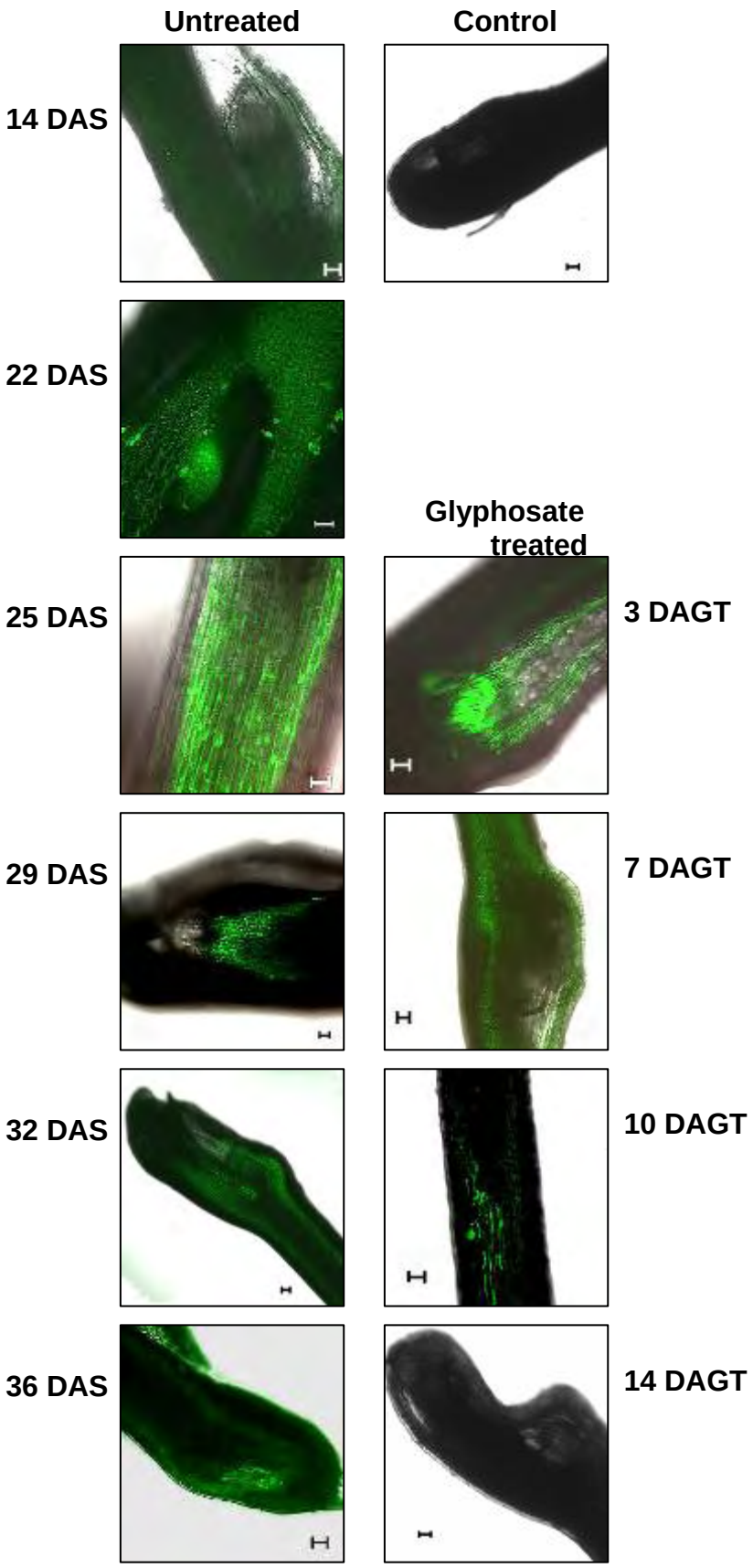


Fig. 13. Green fluorescent protein (GFP) accumulation in *Cuscuta campestris* shoots growing on untreated PEP tobacco plants 14 - 36 days after sowing (DAS); at 22 DAS, the host was treated with glyphosate (36 µg a.e. /plant) and GFP was monitored in the shoots 3 - 14 days after glyphosate treatment (DAGT). Control is a *C. campestris* shoot on a nontransgenic tobacco host (bar 50µm).

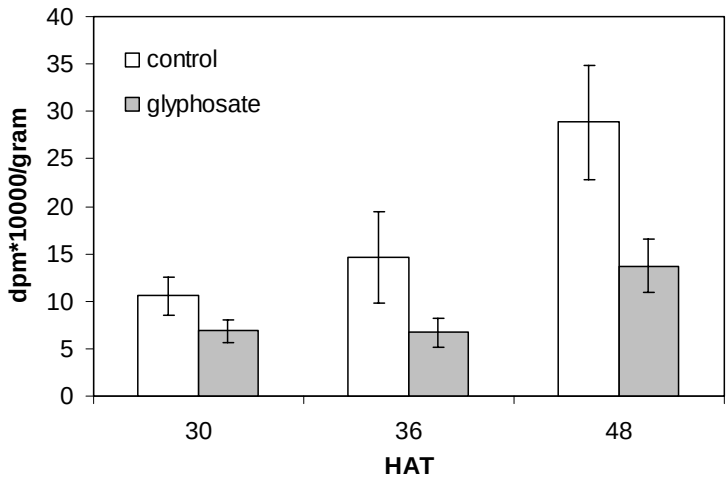


Fig. 14. [^{14}C]sucrose accumulation in *Cuscuta campestris* growing on untreated and glyphosate-treated tobacco 30, 36 and 48 h after treatment (HAT). Vertical bars represent the standard errors of the means.

In parallel, we conducted another series of experiments in our seedling bioassay in Petri dishes using acid scarified field dodder seeds from Or Haner (Israel), sorghum, non-transgenic imidazolinone-resistant (IR) canola (oil seed rape, var. Hylite-289cl IR) and imidazolinone-sensitive (IS, *var*. Hyola-420). Seeds were exposed imazamox mixed in the sand at different concentrations. Germination rate, dodder shoot length and sorghum and oil seed rape root length were measured 7 days after sowing.

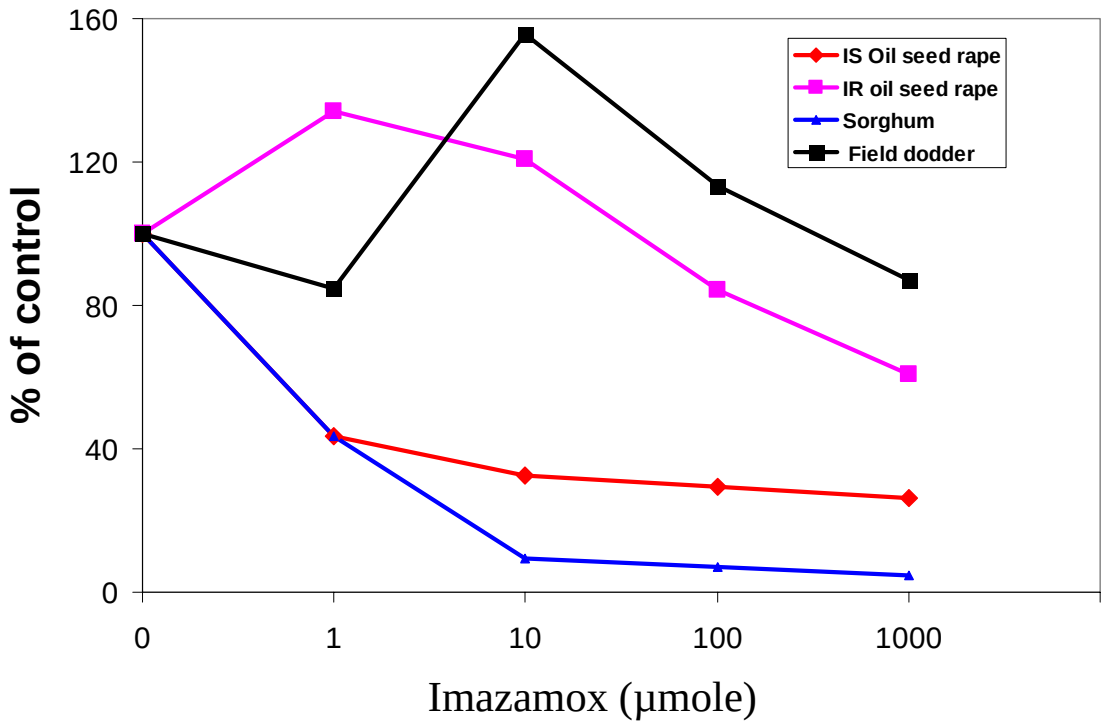


Figure 15. Effect of imazamox on sorghum, imidazolinone-resistant (IR) and –sensitive (IS) canola root elongation and field dodder shoot elongation (seedling bioassay).

The data in Fig. 15 confirmed our previous observation that field dodder can tolerate as high concentrations of AABI herbicide such as imazamox as the IR oil seed rape while the growth of sensitive plants such as sorghum and IS canola is severely inhibited.

Identification of solute movement from host plant to field dodder

It is well established that *C. campestris* serves as a 'super sink' and withdraws all its needs (water, solutes and assimilates) from the host plant. We assumed that although some production of amino acids occurs in the parasite, when the parasite's amino acid biosynthesis (AAB) is inhibited by the AABI herbicides, the parasite could receive 'complementary' supply of amino acids from the host, particularly when the host is not injured by the herbicide. This hypothesis was tested using different pot-grown oil seed rape genotypes (see Table 3 for details) parasitized and non-parasitized with field dodder. Five weeks old oil seed rape plants were decapitated (cut above the first leaf), and their phloem sap was collected. Dodder stems were also cut in places where it was most healthy and their phloem sap was extracted as well.

Table 3. Canola (oil seed rape) genotypes with and without field dodder from which the tissue and phloem sap samples were examined before and after IMI herbicide (imazamox) application.

Genotype and trait	**Abbreviated as**
Canola - imidazolinone resistant - IR	R
Canola - imidazolinone sensitive - IS	S
Canola - IR parasitized with dodder	R-C
Canola - IS parasitized with dodder	S-C
Field dodder - parasitized on IR canola	C on R
Field dodder - parasitized on IS canola	C on S
Flowering canola - IS parasitized with dodder	SF + C
Field dodder-parasitized on flowering IS canola	C on SF

The sap and plant samples were extracted and analyzed after methylation (Fiehn, 2003; Roessner-Tunali et al., 2003) using a GCMS equipped with program that identifies the components of the sap . The peak area of ribitol has served as an internal standard. A Typical output is given in Fig. 16.

Although this study is still in progress, the results of the analysis shows that phloem sap collected from canola plants contain several amino acids with aspartic acid, glutamine and valine being the major ones. It is worthwhile to mention that relatively high rates of shikimic acid were detected confirming our previous observations (Nadler-Hassar and Rubin 2003). Furthermore, imazamox treated plants translocate different mixture of amino acids in the phloem sap, influencing the amino acid supply to the parasite (data not shown).

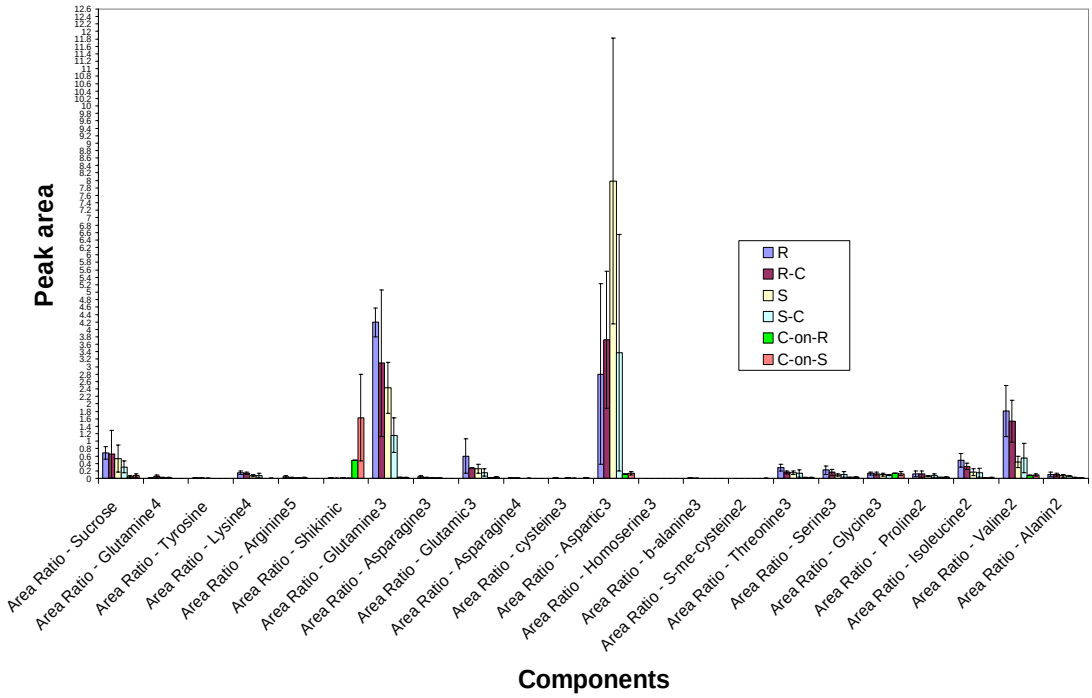


Fig. 16. A typical chromatograph describing the relative content of various components, including amino acids in phloem sap collected from oil seed rape genotype and from field dodder parasitizing them.

Response of canola genotypes to field dodder: During our studies we observed that field dodder "likes" and propagates better on certain canola and tomato genotypes. We hypothesized that penetration and establishment of the parasite vary among genotypes, perhaps because of differences in stem tissue hardiness or robustness. Different canola genotypes (Table 4) were examined. Canola shoots were harvested 7 weeks after planting and biomass of the host and field dodder was determined separately. The results shown in Fig. 17 clearly demonstrate that some genotypes (B, C, E and H) suffer significantly more that the other genotypes (A, D, F and G).

Table 4. Oil seed rape genotypes examined for their sensitivity to field dodder.

Abbreviated as	Genotypes	Trait
A	Hyola-401-S	Glyphosate sensitive
B	Hyola-357-RR	Glyphosate resistant
C	Hyola-420-IS	Imidazolinones sensitive
D	Hylite-289cl-IR	Imidazolinones resistant
E	Phoenix indigo 8-2573 BR	Glufosinate resistant
F	Quantum	None
G	Sary S	None
H	DKL 35-85 RR	Glyphosate resistant

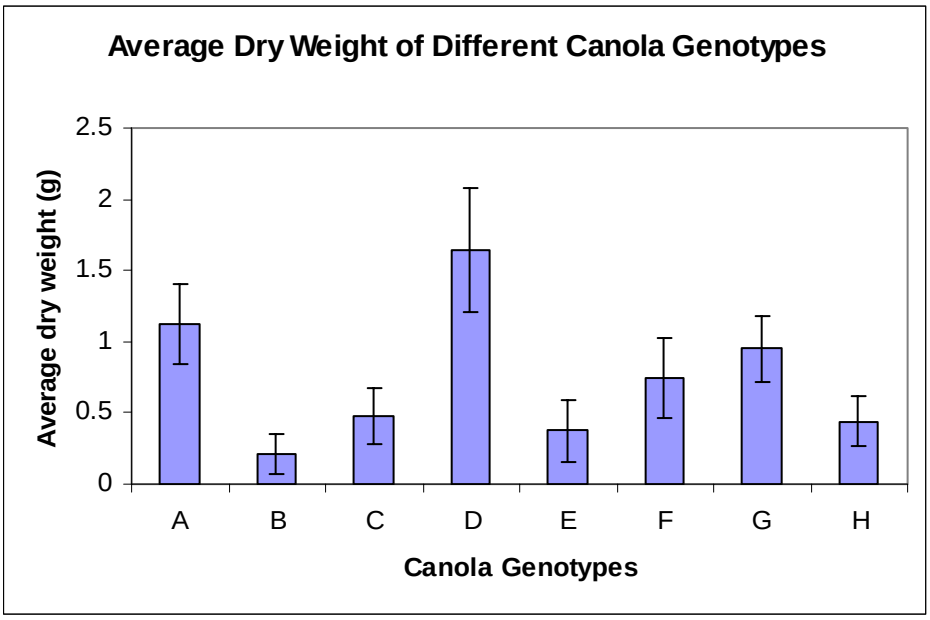


Fig. 17 Shoot biomass production of different oil seed rape genotypes grown in association with field dodder.

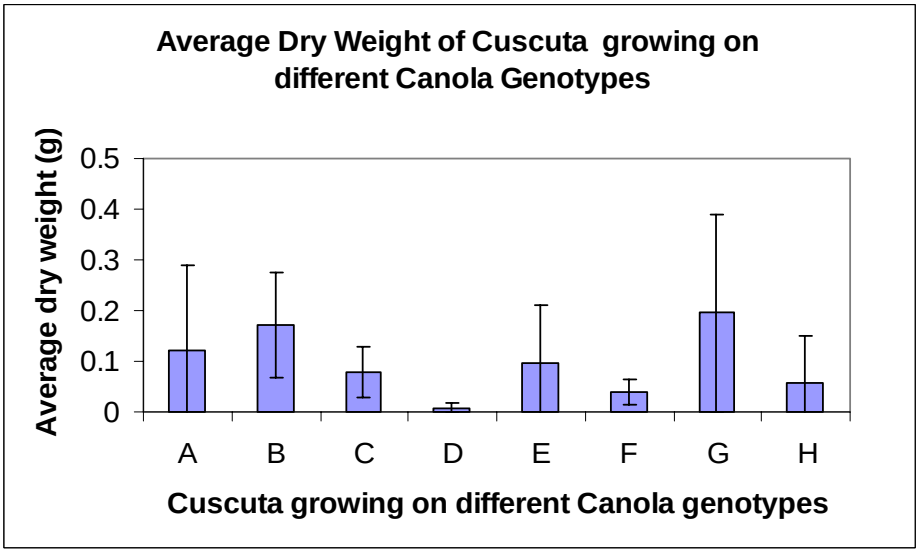


Fig. 18. Field dodder biomass production when grown in association with different genotypes of oil seed rape.

It is a common believe that herbicide tolerant crop technology could provide new management strategies for the control of parasitic plants (Rubin, 1991). The data presented so far raise a real question regarding the potential of using genetically-modified (transgenic or non-transgenic) herbicide-resistant crop plants to combat above-ground parasitic weeds such as field dodder. In a collaborative work with the US group from USDA and Colorado State University we challenged this question. The primary objective of this research was to compare the efficacy of glyphosate, imazamox and glufosinate for control of *C. campestris* while attached to herbicide resistant canola (*Brassica napus* L.).

Herbicide-resistant canola genotypes and one sensitive genotype (Hayola 420) (**WT**) were used to examine the response of attached *C. campestris* to glyphosate (RR-DKL-35-85 - **GRR**), imazamox (Hylite 289 CF - **IR** and 46A76 Pioneer HB, **IR-P**) and glufosinate

(InVigor 2373 - **GLR**). *C. campestris* was allowed to establish on the different canola genotypes before herbicide applications were made.
C. campestris attached to GLR canola was initially affected by glufosinate applications (Fig. 19A), but recovered rapidly (Fig. 19B) and by the end of the experiment had significantly reduced host biomass, thus, the herbicide treatment had very little impact on parasite development. Glufosinate resistance in GLR canola is due to the insertion of a single *bar* gene which encodes an enzyme that rapidly acetylates glufosinate to an inactive metabolite (De Block *et al*., 1987). This resistance mechanism in canola could prevent glufosinate from being translocated and/or accumulated as an intact and active molecule in the parasite. Although attached *Cuscuta* were also sprayed with glufosinate, the amount of herbicide absorbed may not have been sufficient to kill the parasite.

I

Fig. 19: *C. campestris* growing on glufosinate-resistant canola (GLR) treated with 400g ai/ha glufosinate. **A.** growth inhibition. **B.** Recovery of parasite.

Fig 20. *C. campestris* on imidazolinone-resistant canola (IR). **A.** Poor recovery of *C. campestris* on a host treated with 20g ae/ha imazamox. **B.** Host growth restriction by parasite.

Imazamox (40 g ae/ha) completely inhibited *C. campestris* growth on IR canola (Fig. 20A): however, in spite of the initial damage, the parasite was able to reduce the biomass of the IR oilseed rape up to 80%. This occurred when the parasite attachment encircled the canola stem, causing a severe restriction of stem development that lasted even after the parasite had died

(Fig. 20B). On the other hand, IR canola contains two modified ALS genes that encode for imidazolinone resistant enzymes (Shaner *et al*., 1996). When imazamox is applied to IR canola, the herbicide is not readily metabolized and can rapidly accumulate in the attached *C. campestris*, which acts as a "super sink". Thus, the parasite is continuously exposed to a high dose of the herbicide.

Glyphosate applied at high rates to GRR canola significantly reduced *C. campestris* biomass (Fig. 21A); however, 34 DAT most of the herbicide-treated *Cuscuta* plants maintained viable and recovering apexes (Fig. 21B-C) and by 90 DAT the host plants were demolished whereas the parasite set seeds (Fig. 21D).

Fig 21. The response of *C. campestris* growing on a glyphosate-resistant canola (GRR) host to treatment with 750g ai/ha glyphosate. **A.** 18 DAT **B.** 29 DAT **C.** 43 DAT **D.** 90 DAT.

In vivo shikimate and ALS assays confirm that *C. campestris* has active ALS and EPSPS (Nadler-Hassar & Rubin, 2003) suggesting that the tolerance of seedlings to glyphosate and imazamox is not related to an altered target site. These data support our theory that the interaction between the *Cuscuta* and host may trigger different processes in the parasite.

Glyphosate-resistant canola (GRR) contains two genetic modifications. It carries the CP4 EPSPS gene that encodes a glyphosate-resistant EPSPS and a second gene that encodes for a glyphosate degrading enzyme - glyphosate oxidoreductase (GOX). This gene was isolated from a common soil bacterium *Achromobacter* sp. and rapidly degrades glyphosate to non-toxic products, aminomethylphosphonic acid (AMPA) and glyoxylate (Canadian Food Inspection Agency, 1996; Padgette *et al*., 1996). When glyphosate is applied to GRR canola, part of it will be metabolized to inactive metabolites. This will reduce the amount intact

glyphosate that can be translocated to the attached *C. campestris*. Thus, the parasite would be exposed to less herbicide, resulting in reduced control.

C. campestris appeared to be very compatible with the GRR canola variety and this may have attributed to the parasite ability to obtain enough nutrients from the host to recover from glyphosate treatments. Our previous observations have shown that *C. campestris* was more likely to recover from herbicide treatments while attached to GRR sugar beet (Nadler-Hassar and Rubin, 2003), then if attached to GRR soybean. These differences could be attributed to the quality of attachment between the host and parasite. The importance of good establishment and compatibility between the host and parasite has also been reported to enhance the translocation of labelled nitrogen from the host to *C. campestris* (Koch *et al*., 2004), to increase the likelihood that *C. gronovii* would mature on a host (Schoolmaster, 2005) and ensure the survival of *C. subinclusa* during hot summer (Kelly, 1990).

Our results suggest that the most important factor in determining the efficacy of AABI herbicides to control *Cuscuta* could be the quality of attachment between the host and parasite. The strong attachment between *C. campestris* and GLR oilseed rape enabled the parasite to overcome herbicide injury and to recover from high glufosinate applications. These results raise question regarding the effectiveness of using AABI-resistant tomato or lucerne to control *C. campestris*. Both crops are sensitive to the parasite and it has been reported that one application of glyphosate was not sufficient to control *C. campestris* in lucerne (Dawson *et al*., 1994).

The reason why *C. campestris* has a lower compatibility to IR canola is not yet known but evidence collected so far in our laboratory indicates that difference in the lignin content in the stem and petiole may influence the ability of *C. campestris* to establish good connection with the host plant. The results of this research suggests that developing parasite resistant crops such as tomato with lower compatibility (Goldwasser *et al*., 2001) or hyper sensitive response might be better alternatives to and possibly compatible with herbicide-resistant crops. Each parasite-crop system needs to be evaluated on a case by case basis to determine the most economical system for managing the parasite.

F. Impact, Relevance and Technology Transfer:

The findings of our research are already implemented in Kazakhstan as the experiments were conducted in commercial fields with farm managers involved in executing of the treatments and data collections.
Based on our results, farmers in Israel and Kazakhstan are better informed on herbicides capacity to control field dodder. The Co-PI has published several papers in Russian so the information is or will be available in the neighboring countries such as Kirgizstan, Uzbekistan and alike.
The fact that our project was "the major internationally funded project" in the Institute of Plant Protection (KIPP), with research activities spreading all over the major agricultural regions, contributed to the elevated prestige and the "status" of the department of weed science in KIPP and the country.
Ms Aijan Jusupova, a M.Sc. student from the Al-Farabi Kazakh National University, School of Biology, Department of Genetics and Molecular Biology - has conducted her thesis research with the Co-PI. This study was published in two papers (see below). Ms Jusupova continues her studies for a Ph.D. Program at the same university where the Israeli PI was nominated as her External supervisor. She will be visiting the FAR laboratory for two months training starting October 2007. She will investigate some molecular aspects involved in *Cuscuta* growth.
Another young scientist, Dr. Zhanna Issina from KIPP has spent two periods of training in the FAR laboratory. She joined our group, gained experience in various laboratory, greenhouse and field methods which were instantly employed upon her return to KIPP (see her attached letter, **Appendix III**). The Co-PI Prof. Zharasov has visited FAR towards the final stages of the project and took an active part in summarizing the experiments and the preparation of the final report.
In FAR, Dr. Talia Nadler-Hassar has completed her Ph.D. program partially funded by the project. Ms. Orly also participated in the project as a M.Sc. student – she is about to submit her thesis. Both of them were instrumental in assisting the trainee Dr. Issina during her stay in our lab, helping her learn the theories and practicum in field dodder research.

G. Project Activities/Outputs:

Meetings – There were several meeting between the Co-PI and PI:

1. The PI visited the KIPP (July 6 to 14, 2003). As described in **Appendix IV**, the meeting was very important in terms of meeting with potential trainees, administration and overcoming local problem that interfere with the smooth progress of the project.
2. We met while both the PI and Co-PI attended the 15th IPPC in Beijing, China during May 2004. We discussed our project results and planned future work.
3. During July 2006 the Co-PI visited FAR and joined the trainee Dr. Zhanna Issina who was already in Israel for her second training period. We summarized the activities and a draft of the Final report was established. During his visit, they met with Mrs. Elinor Slater and her accounting team and was briefed about the financial situation of the project.

Training:

1. Ms. Aijan Jusupova was trained in Alamty during the PI visit to KIPP (July 2003). She joined us to all field trials site, and was exposed to preparation of field and laboratory and greenhouse experiments. She will visit FAR early October 2007 until end of the year for training in molecular work as part of her graduate studies.

2. Dr. Zhanna Issina, who joined Prof Zharasov group on 2003, has a previous experience in phytopathology but not in weed science. She visited our laboratory November 10 to December 26, 2004. She worked with my graduate students, learned how we deal with field dodder with and without host plants. She also exposed the professional literature in English. For more details on her training and accomplishnments please see **Appendix III**.
3. Dr. Issina has visited us again during 31st of May until 26th of July 2006. This second period was by far more productive for her in terms of gaining experience in laboratory and greenhouse work. She also was instrumental in getting the draft of the Kazakh final report.

- **List of publications.**

Papers published from this project by the Kazakh group:

1. Zharasov Sh. U. 2004. Ecology and Sustainable Control of the Parasitic Weed Field Dodder (*Cuscuta campestris*) CA-20-006. Abstracts. *Proceedings of the 15th International Plant Protection Congress (IPPC). Beijing. China*. May 11-16. 2004. pp. 753
2. Zharasov Sh. U. 2004. Quarantine Weeds and Fundamentals of Weed Control. *Reports of National Science Academy*. pp. 70-74.
3. Zharasov Sh. U. 2004. Quarantine Weeds of Kazakhstan. In: Chemical Control: Conditions and Perspectives for Increase Ecology. *St. Petersburg*. pp. 113-115.
4. Zharasov Sh. U. 2004. Ecology and Control of Quarantine Weeds in Kazakhstan. *International Conference "Strategy of Science Guarantee in Agriculture". The Reality and Perspective*. Almaty. Book 2. pp. 179-180.
5. Zharasov Sh. U. 2005. Effect of Field Dodder on the Seedling of Agricultural Crops. *Modern Problem of Quarantine and Plant Protection*. pp. 464-469.
6. Zharasov Sh.U. and Jusupova A.I. 2005. Phytochemical Determination of Some Groups of Biological Active Substances of Field Dodder. *Modern Problem Quarantine and Plant Protection*. pp.428-432.
7. Zharasov Sh.U. and Jusupova A. 2005. Field Dodder Control in the Alfalfa and Herbicide Treatment. In: *Modern Problems of Quarantine and Plant Protection. Almaty*. pp. 470-472.

Papers from this project published by the Israeli group:

1. Nadler-Hassar, T. and B. Rubin (2003). Natural tolerance of *Cuscuta campestris* Yunk. to herbicides inhibiting amino acid biosynthesis. *Weed Research* **43**, 341-347.
2. Nadler-Hassar, T., Goldshmidt, A., B. Rubin and S. Wolf (2004). Glyphosate inhibits the translocation of GFP and sucrose from a transgenic tobacco host to *Cuscuta campestris* Yunk. *Planta* **219**, 790-796.
3. Nadler-Hassar T, Shaner D, Nissen S, Rubin B, and Westra P (2007) Are herbicide resistant crops the answer to controlling *Cuscuta*? Submitted for publication.

Papers published in Proceedings:

1. Nadler-Hassar T and Rubin B (2003) Glyphosate inhibits the translocation of the reporting protein (GFP) and sucrose from a transgenic tobacco to field dodder. Proceedings of the 17th Israeli Weed Science Conference. Rehovot, Israel p. 13.

2. Nadler-Hassar T, Zygier L and Rubin B. (2004) Response of *Cuscuta* spp. and *Orobanche* spp. to herbicides inhibiting amino acid biosynthesis. *Proceedings of the COST Action 849 Meeting , Nitra, Slovakia*, June 2004.
3. Korber O, Nadler-Hassar T and Rubin B (2007) Assimilates and amino acids movement in field dodder and its host plants. Proceedings of the 19th Israeli Weed Science Conference. Rehovot, Israel p. 2.
4. Hassar-Nadler T, Zygier L, Korber O, Shaner D and Rubin B (2005) The Mode of Action of Herbicides in Host-Parasite Interaction. *Proceedings of the COST Action 849 Meeting , Newe-Ya'ar, Israel*, June 2005
5. Nadler-Hassar T, Shaner D, Nissen S, Westra P and Rubin B (2006) Evidence for anabolic and catabolic forms of ALS in dodder. *Proceedings of the American Weed Science Society*, February 2006.
6. Rubin B, Zygier L, Korber O and Hassar-Nadler T (2006) Effect of herbicides inhibiting amino acid biosynthesis on *Cuscuta* spp. and *Orobanche* spp. *Proceedings of the COST Action 849 Meeting , Oeiras-Lisbon, Portugal*, November 2006

H. Project Productivity:

The project accomplished most of the proposed goals. The technology was transferred to the KIPP employees, young scientists from KIPP were trained in Israel. The Kazakh farmers have now better means to combat the parasite, and the scientific personnel at KIPP better equipped to meet challenges. Prof. Zharasov the Kazakh leader of the project is close to retirement, but thanks to this project, the knowledge and skills are at least partially,transferred to the younger generation who will continue to develop better ways to combat parasitic weeds.

I. Future Work:

We believe that the connections formed between the KIPP and FAR will continue in the future. As mentioned above, a young scientist from Kazakhstan will visit the Israeli laboratory for training in modern biological methods. This visit is funded by the Al-Farabi Kazakh National University, School of Biology, Department of Genetics and Molecular Biology. The PI was invited to serve as Guest Professor during 2008, lecturing and discussing selected scientific topics with faculty and students.

J. Literature Cited

Amerhein N, Deus B, Gehrke P and Steinrucken HC (1980) The site of the inhibition of the shikimate pathway by glyphosate II. Interference of glyphosate with chorismate formation *in vivo* and *in vitro*. *Plant Physiology* **66**, 830-834.

Arstangaliev S A and Ramasanov E P (1977) Flora of Kazakhstan. Alma-Ata, (In Russian).

Baerson SR, Damian JR, Tran M, Feng Y, Biest NA and Dill GM (2002) Glyphosate-resistant goosegrass. identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase. *Plant Physiology* **129**, 1265-1275.

Bestman HD, Devine MD and Vanden Born WH (1990) Herbicide chlorsulfuron decreases assimilate transport out of treated leaves of field pennycress (*Thlaspi arvense* L.) seedlings. *Plant Physiology* **93**, 1441-1448.

Bewick TA, Binning LK and Balke NE (1991) Absorption and translocation of glyphosate by carrot infected by swamp dodder. *Journal of the American Society of Horticultural Science* **116**, 1035-1039.

Cromartie TH, & Polge ND (2000) An improved assay for Shikimic acid and its use as a monitor for the activity of sulfosate. *WSSA Abstracts* **40**, #291.

Dawson JH (1987) *Cuscuta* (Convolvulaceae) and its control. In: *Proceedings, 4th International Symposium on Parasitic Flowering Plants* (eds. HC Weber and W Forstreuter), 137-149, Marburg, Germany.

Dawson JH, Musselman LJ, Wolswinkel P and Dorr I (1994) Biology and control of *Cuscuta*. *Review Weed Science* **6**, 265-317.

De block M, Botterman J and Vemderwiele M (1987) Engineering herbicide resistance in plants by expression of a detoxifying enzyme. *EMBO Journal* **6,** 2513-2518.

Devine MD, Duke SO and Fedtke C (1993) Inhibition of amino acid biosynthesis. In: *Physiology of Herbicide Action*, 251-294 Prentice-Hall Inc., New Jersey, USA.

Dinelli G, Bonetti A and Tibiletti E (1993) Photosynthetic and accessory pigments in *Cuscuta campestris* Yunker and some host species. *Weed Research* **33**, 253-260.

Dörr I (1987) The haustorium of *Cuscuta*- new structural results. In: *Proceedings, 4th International Symposium on Parasitic Flowering Plants* (eds. HC Weber and W Forstreuter), 163-173, Marburg, Germany.

Feinbrun-Dothan, N. and A. Danin (1991). *Analytical Flora of Eretz Israel*, 1040 pp. CANA Publishing House, Ltd. Jerusalem, Israel.

Fer A (1984) Physiological approach to the chemical control of *Cuscuta*: experiments with ^{14}C-labeled herbicides. In: *Proceedings, 3rd International Symposium on Parasitic Weeds* (eds. C Parker, LJ Musselman, RM Polhill and AK Wilson), 164-174, ICARDA, Aleppo, Syria.

Fiehn O (2003) Metabolic networks of *Cucurbita maxima* phloem**.** *Phytochemistry* **62**, 875-886.

Geiger DR and Bestman HD (1990) Self-limitation of herbicide mobility by phytotoxic action. *Weed Science* **38**, 324-329.

Geiger DR, Shieh W-J and Fuchs MA (1999) Causes of self-limited translocation of glyphosate in *Beta vulgaris* plants. *Pesticide Biochemistry and Physiology* **45**, 124-133.

Goldwasser Y, Lanini WT and Wrobel RL (2001) Tolerance of tomato varieties to lespedeza dodder. *Weed Science* **49**, 520-523.

Gresshoff PM (1979) Growth inhibition by herbicide glyphosate and reversal of its action by phenylalanine and tyrosine *Escherichia coli*, *Chlamydomonas reinhardi*, *Arabidopsis thaliana*. *Australian Journal of Plant Physiology* **6**, 177-185.

Hall LM and Devine MD (1993) Chlorsulfuron inhibition of phloem translocation in chlorsulfuron-resistant and -susceptible *Arabidopsis thaliana*. *Pesticide Biochemistry and Physiology* **45**, 81-90.

Haupt S, Oparka KJ, Sauer N and Neumann S (2001) Macromolecular trafficking between *Nicotiana tabacum* and the holoparasite *Cuscuta reflexa*. *Journal of Experimental Botany* **52**, 173-177.

Hibberd JM, Bungard RA, Press MC, Jeschke WD, Scholes JD and Quick WP (1998) Localization of photosynthetic metabolism in the parasitic angiosperm *Cuscuta reflexa*. *Planta* **205**, 506-513.

Hickman, J. C. (1993). *The Jepson Manual of Higher Plants of California*. 1400p University of California Press, Berkeley.

Holm L, Doll J, Holm E, Panch J and Herberger J (1997) *World Weeds: Natural Histories and Distribution*. John Wiley and Sons, New York, USA.

Jaworski EG (1972) Mode of action of N-phosphonomethylglycine: inhibition of aromatic amino acid biosynthesis. *Journal of Agricultural and Food Chemistry* **20**, 1195-1205.

Jescke WD, Baumel P, Rath N, Czygan F-C and Proksch P (1994) Modeling of the flows and partitioning of carbon and nitrogen in the holoparasite *Cuscuta reflexa* Roxb. and its host *Lupinus albus* L. II. Flows between the host and parasite and within the parasitized host. *Journal of Experimental Botany* **45**, 801-812.

Joel DM, Kleifeld Y, Losner-Goshen D, Herzlinger G and Gressel Y (1995) Transgenic crops against parasites. *Nature* **374**, 220-221.

Kanampiu FK, Ransom JK and Gressel J (2001) Imazapyr seed dressings for *Striga* control on acetolactate synthase target-site resistant maize. *Crop Protection* **20,** 885–895.

Kelly CK (1990) Plant foraging: a marginal value model and coiling response in Cuscuta subinclusa. *Ecology* **71**, 1916-1925.

Kim S and Vanden Born WH (1996) Chlorsulfuron decreases both assimilate export by source leaves and import by sink leaves in Canola (*Brassica napus* L.) seedlings. *Pesticide Biochemistry and Physiology* **56**, 141-148.

Koch AM, Binder C and Sanders IR (2004) Does the generalist parasitic plant *Cuscuta campestris* selectively forage in heterogeneous plant communities? *New Phytologist* **162**, 147-155.

Lee TT and Starratt AN (1989) Phenol-glyphosate interaction: effects on IAA metabolism and growth of plants. In: *Adjuvants and Argrochmicals vol. 1, Mode of Action and Physiological Activity* (eds. PNP Chow, CA Grant, AM Hinshalwood and E Simundson), 35-40 CRC press, Boca Raton, Florida, USA.

Liu ZQ and Fer A (1990) Effect of a parasite (*Cuscuta lupuliformis* Krock.) on the redistribution of two systemic herbicides applied on a legume (*Phaseolus aureus* Roxb.). *Comptes Rendus de L'Academie des Sciences, Series 3, Science de la Vie* **310**, 333-339.

Liu ZQ, Fer A and Lecocq M (1991) Imazaquin; a promising herbicide for the control of dodder (*Cuscuta* spp.) in soybean (*Glycine* max). *Weed Research* **31**, 33-40.

Nadler-Hassar T and Rubin B (2003) Natural tolerance of *Cuscuta campestris* Yunk. to herbicides inhibiting amino acid biosynthesis. *Weed Research* **43**, 341-347.

Nadler-Hassar, T., Goldshmidt, A., B. Rubin and S. Wolf (2004). Glyphosate inhibits the translocation of GFP and sucrose from a transgenic tobacco host to Cuscuta campestris Yunk. *Planta* **219**: 790-796

Nandula VK, Foy CL and Orcutt DM (1999) Glyphosate for *Orobanche aegyptiaca* control in *Vicia sativa* and *Brassica napus*. *Weed Science* **47**, 486-491.

Nir E, Rubin B and Zharasov SW (1996) On the biology and selective control of field dodder (*Cuscuta campestris*). In: *Advances in Parasitic Plant Research, 6th International Parasitic Weed Symposium* (eds. MT Moreno, JI Cubero, D Berner, D Joel, LJ Musselman and C Parker), 809-816, Junta de Andalusia Cordoba, Spain.

Oparka KJ, Roberts AG, Boevink P, Santa Cruz S, Roberts I, Pradel KS, Imlau A, Kotlizky G, Sauer N, Epel B (1999) Simple, but not branched, plasmodesmata allow the nonspecific trafficking of proteins in developing tobacco leaves. *Cell* **97**, 743-754.

Padgette SR, Re DB, Barry GF, Eichholtz DE, Dalanay X, Fuchs RL, Kishore GM, and Fraley RT (1996) New weed control opportunities: development of soybeans with a Round Ready™ gene. S. O. Duke, ed. *Herbicide-Resistant Crops: Agricultural, Environmental, Economic, Regulatory, and Technical Aspects.* 53-84 Boca Raton, FL: CRC Press.

Parker C and Riches CR (1993) *Parasitic Weeds of the World: Biology and Control*. CAB International, Wallingford, UK.

Press MC, Graves GR and Stewart GR (1990) Physiology of the interaction of angiosperm parasites and their higher plant hosts. *Plant Cell and Environment* **13**, 91-104.

Ray TB (1984) Site of action of chlorsulfuron. Inhibition of valine and isoleucine biosynthesis in plants. *Plant Physiology* **75**, 827-831.

Roessner-Tunali U, Hegemann B, Lytovchenko A, Carrari F, Bruedigam C, Granot D, and Fernie AR (2003) Metabolic profiling of transgenic tomato plants overexpressing hexokinase reveals that the influence of hexose phosphorylation diminishes during fruit development. *Plant Physiology* **133**, 1-16.

Rubin B (1991) Herbicide resistance in weeds and crops, progress and prospects. In: *Herbicide Resistance in Weeds and Crops* J. C. Caseley, G. Cussans and R. Atkin eds. 387-414 Butterworths-Heinemann, UK.

Schoolmaster DR Jr. (2005) *Impatiens capensis* (Balsaminaceae) Meerb. Is a necessary nurse host for the parasitic plant *Cuscuta gronovii* (Cuscutaceae) Willd. Ex J.A Schultes in southeastern Michigan wetlands. *American Midland Naturalist* **153,** 33-40.

Seefeldt SS, Jensen JE and Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships. *Weed Technology* **9**, 218-227.

Shaner DL. Bascomb NF, and Smith W (1996) Imidazolinone-resistant crops: selection, characterization and management. 143-157 S. O. Duke, ed. *Herbicide-Resistant Crops: Agricultural, Enviornmental, Economic, Regulatory, and Technical Aspects.* Boca Raton, FL: CRC Press.

Singh BK & Shaner DL (1998) Rapid determination of glyphosate injury to plants and identification of glyphosate-resistant plants. *Weed Technology* **12**, 527-530.

Tranel PJ and Wright TR (2002) Resistance of weeds to ALS inhibiting herbicides: what have we learned? *Weed Science* **50**, 700-712.

Tsivion Y (1979) The regulation of the association of the parasitic plant *Cuscuta campestris* with its hosts. PhD Thesis, The Hebrew University of Jerusalem, Israel.

Vaughn KC (2002) Attachment of the parasitic weed dodder to the host. *Protoplasma* **219**, 227-237.

Vaughn KC (2003) Dodder hyphae invade the host: a structural and immunocytochemical characterization. *Protoplasma* **220**, 201-207.

Weinberg T, Lalazar A and Rubin B (2003) Effects of bleaching herbicides on field dodder *(Cuscuta campestris*). *Weed Science* **51**, 663-670.

Wolswinkel P (1984) Recent developments in phloem transport research. The parallel between the site of attachment of *Cuscuta* and developing seeds of legumes. *Proceedings, 3rd International Symposium on Parasitic Weeds* (eds. C Parker, LJ Musselman, RM Polhill and AK Wilson), 156-163, ICARDA, Aleppo, Syria.

Wolswinkel P (1986) Phloem unloading in stem parts parasitized by *Cuscuta*. In: *Phloem Transport* . J Cronshow, WJ, Lucas and RT Giaquinta eds. 239-246 Alan R Liss Inc., New York, USA,

Wolswinkel P, Ammerlaan A and Peters HFC (1984) Phloem unloading of amino acids at the site of attachment of *Cuscuta europaea*. *Plant Physiology* **75**, 13-20.

Yuan CI, Chaing MY and Chen YM (2002) Triple mechanisms of glyphosate-resistance in a naturally occurring glyphosate-resistant plant *Dicliptera chinensis. Plant Science* **163**, 543-554.

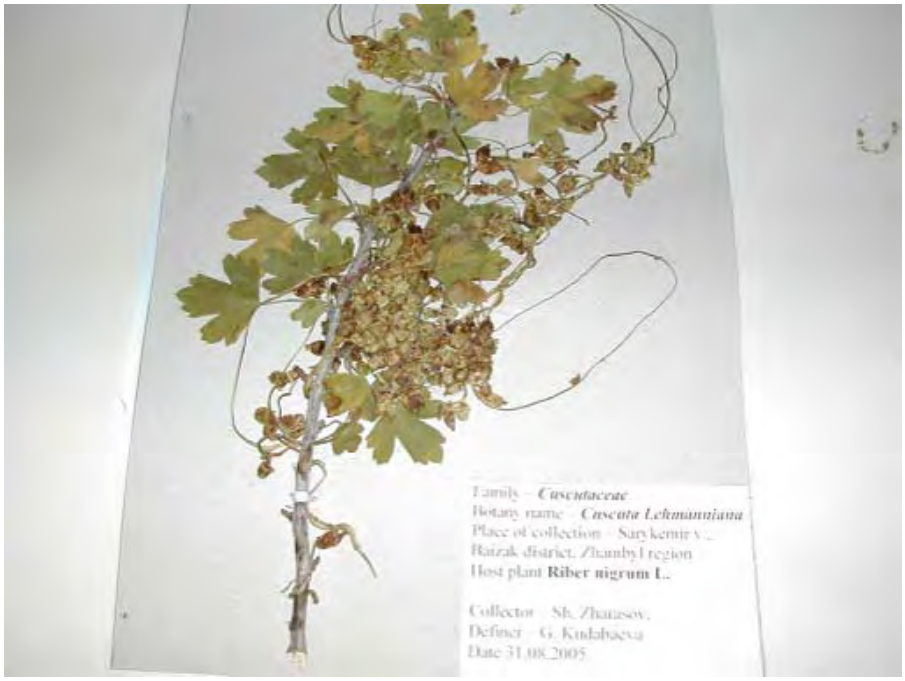
Appendix I.

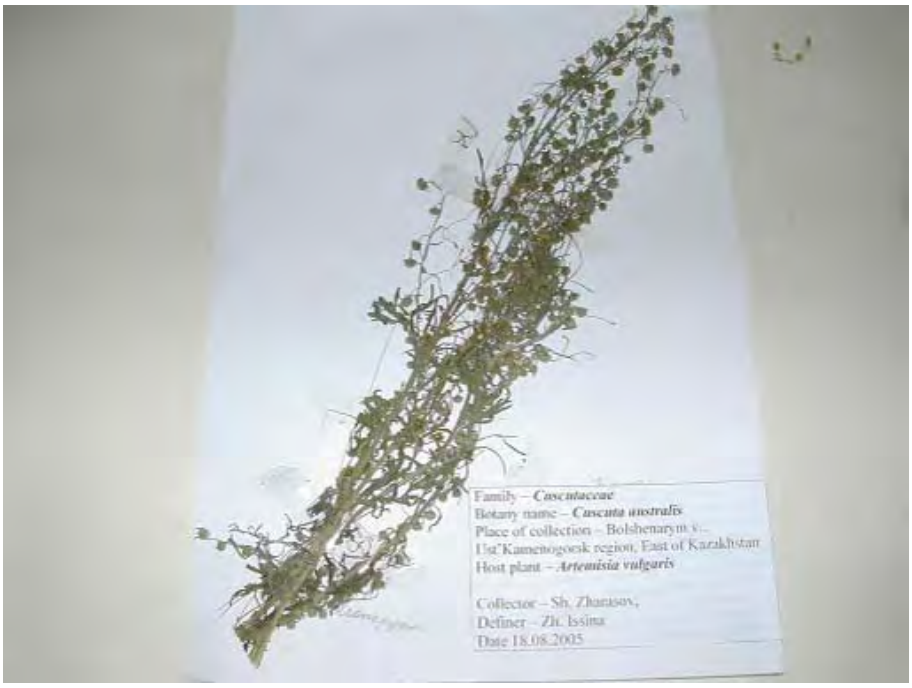
Dodder populations collected in different regions of Kazakhstan as identified by Dr. Kudabaeva.

Species	**Region**	**District**	**Village**	**Crop**
C. campestris	Almaty	Talgar	Panfilova	Sugar beet
C. campestris	Almaty	Talgar	Ray of Orient	Red beet
C. monogyna	Almaty	Talgar	Kyzyl tu	Bushes, *Malva*
C. campestris	Almaty	Talgar	Luch Vostoka	Red beet
C. campestris	Almaty	Karasai	KIZ	Roadside
C. campestris	Almaty	Ili		Safflower
C. campestris	South Kazakh.	Sairam	Aksu	Roses
C. campestris	East Kazakh.	Zaisan		Alfalfa
C. campestris	East Kazakh.	Zaisan	Karabulak	
C. campestris	East Kazakh.	Zaisan	Kensai shilikty	
C. monogyna	East Kazakh.	Sarterek Balykbai		
C. lupuliformis	East Kazakh.	Sarterek	Karashuki	Roses
C. campestris	East Kazakh.	Mailibai	Kensai	Alfalfa
C. campestris	East of Kazakh.	Mailibai	Kensai	*Convulvulus*
C. campestris	Kostanai	Arkalyk		*Matricaria*
C. campestris	Kostanai	Arkalyk		*Chenopodium*
C. lupuliformis	Astana	Kurgaldzhinskii		*Lonicera*
C. lupuliformis	Astana	Kurgaldzhinskii		*Lathyrus*
C. lupuliformis	Astana	Kurgaldzhinskii		*Rubus*
C. lupuliformis	Astana	Kurgaldzhinskii		*Salix*
C. campestris	Astana	Kurgaldzhinskii		*Convolvulus*
C. campestris	Astana	Kurgaldzhinskii		Artemisia
C. campestris	Aktobe	Kribo lake		*Polygonum*
C. campestris	Aktobe	Kribo lake	Novol'dzhinski i	
C. campestris	South Kazakh.	Tulkubas	Birlik	Onion
C. campestris	South Kazakh.	Tulkubas	Birlik	Alfalfa
C. campestris	South Kazakh.	Tulkubas	Balykty	*Matricaria*
C. campestris	South Kazakh.	Tulkubas	Shakpak	Clover

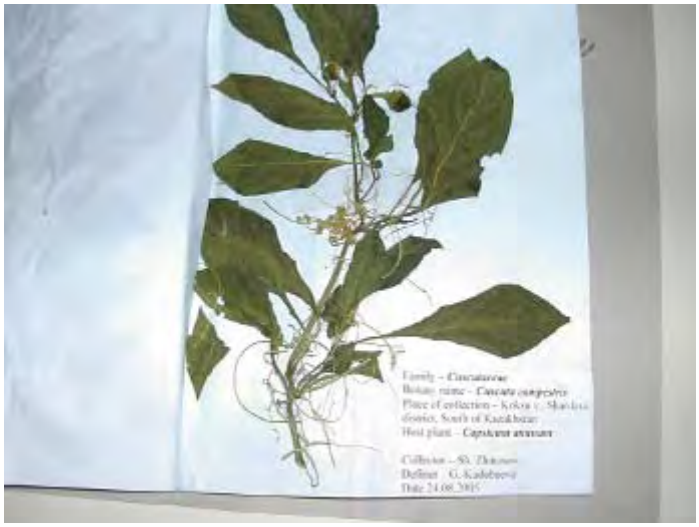
C. campestris	South Kazakh.	Tulkubas	Vannovka	*Polygonum aviculare*
C. campestris	South Kazakh.	Tulkubas	Vannovka	*Cirsium arvense*
C. australis	South Kazakh.	Tulkubas	Vannovka	*Robinia pseudoacacia*
C. campestris	South Kazakh.	Asyk Ata		Tomato
C. campestris	South Kazakh.	Mankent		Cucumber (
C. campestris	South Kazakh.	Akbulak		Tobacco
C. monogyna	South . Kazakh.	Akbulak		Smooth leaved elm (*Ulmus foliacea*)
C. campestris	South . Kazakh.	Kainarbulak		*Alhagi Pseudalhagi*
C. campestris	South . Kazakh.	Aksunet		*Aster sedifolius*
C. campestris	South Kazakh.	Karasu		*C. arvense* L.
C. campestris	South Kazakh.	Karasu		Carrot
C. monogyna	Zhambyl	Taraz		Black currant
C. monogyna	Zhambyl	Taraz		Sorghum
C. monogyna	Zhambyl	Taraz		Spurge
C. Lemanniana	Zhambyl	Taraz		Maple (*Acer platanoides*)
C. campestris	Zhambyl	Taraz		*Solanum rostratum*
C. campestris	Zhambyl	Baizak		*Datura metel* L.
C. campestris	Zhambyl	Baizak		Black currant
C. campestris	Zhambyl	Baizak		Alfalfa
C. lupuliformis	Zhambyl	Baizak		White poplar (*Populus alba*)
C. campestris	Zhambyl	Baizak		*Polygonum aviculare*

Photos prepared for the identification of *Cuscuta* spp. Infesting crops and habitats in Kazakhstan

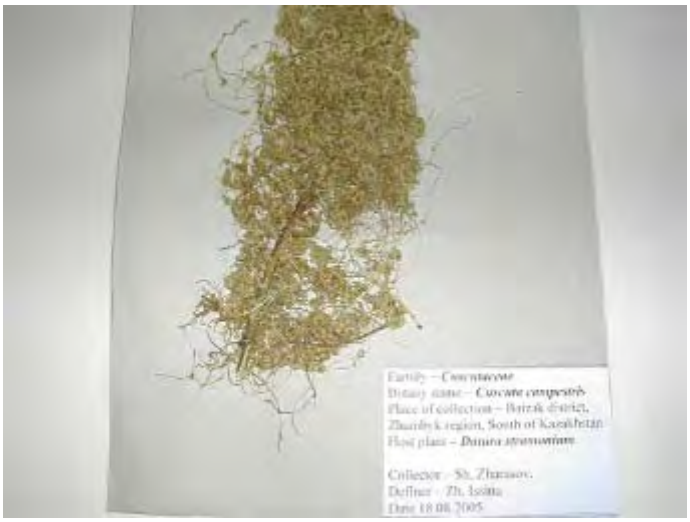




Family – Cuscutaceae
Botany name – Cuscuta lupuliformis
Place of collection – Taldy city, Zhambyl region
Species was collected on Acer platanoides.
Collector – Sh. Zhumaev,
Definer – G. Kodabaeva
Date 31.08.2005


Family – Cuscutaceae
Botany name – Cuscuta campestris
Place of collection – Kokta v., Shardara
district, South of Kazakhstan
Host plant – Capsicum annuum
Collector – Sh. Zhumaev
Definer – G. Kodabaeva
Date 24.08.2005

Family – Cuscutaceae
Botany name – Cuscuta campestris
Place of collection – Koseb v., Shardara
district, South of Kazakhstan
Host plant – Solanum melongena
Collector – Sh. Zhumaev,
Definer – G. Kodabaeva
Date 24.08.2005


Family – Cuscutaceae
Botany name – Cuscuta compestris
Place of collection – Baizak district,
Zhambyl region, South of Kazakhstan
Host plant – Datura stramonium
Collector – Sh. Zhanaev,
Definer – Zh. Issina
Date 18.08.2005

Family – Cuscutaceae
Botany name – Cuscuta australis
Place of collection – Daryl v., Baizak district,
Zhambyl region
Host plant Datura metel L.
Collector – Sh. Zhanaev,
Definer – G. Kadzhaeva
Date 31.08.2005

Family – Cuscutaceae
Botany name – Cuscuta monogyna
Place of collection – Shaimaev v., Ordzhonikidze
district, South of Kazakhstan
Host plant – Prunus domestica
Collector – Sh. Zhanaev,
Definer – G. Kadzhaeva
Date 27.08.2005

Family – Cuscutaceae
Botany name – Cuscuta campestris
Place of collection – Aydarkul r., Baydibek district, South of Kazakhstan
Host plant – Allium inscscum
Collector – Sh. Zhumaev,
Definer – G. Kozubaeva
Date 23.08.2005

Family – Cuscutaceae
Botany name – Cuscuta campestris
Place of collection – Kokaral r., Baydibek district, South of Kazakhstan
Host plant – Anethum graveolens
Collector – Sh. Zhumaev,
Definer – G. Kozubaeva
Date 24.08.2005

Family - Cucurbitaceae
Botany name - Cucumis lehmannsianus
Place of collection - Kazakstan r.,
Baizak district, Zhambyl region
Host plant Bilbo nigrum L.
Collector - Sh. Zhanaev,
Definer - G. Kudabaeva
Date 31.08.2005

Family – Cucurbitaceae
Botany name – Cucurbita moschata
Collector – Sh. Zhanaev
Definer – G. Kudabaeva
Date 21.08.2007

Family – Cucurbitaceae
Botany name – Cucurbita moschata
Collector – Sh. Zhanaev
Definer – G. Kudabaeva

Appendix II

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

T NADLER-HASSAR & B RUBIN
Faculty of Agricultural, Food and Environmental Quality Sciences, R. H. Smith Institute of Plant Sciences and Genetics, The Hebrew University of Jerusalem, Rehovot 76100, Israel

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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-enolpyruvyls-hikimate-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*

Correspondence: B Rubin, Faculty of Agricultural, Food and Environmental Quality Sciences, R. H. Smith Institute of Plant Sciences and Genetics, The Hebrew University of Jerusalem, Rehovot 76100, Israel. Tel: (+972)-8-9489248; Fax: (+972)-8-9362083; E-mail: rubin@agri.huji.ac.il

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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, 480 g a.i. L^{-1}, 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

	I_{50}							
Herbicide	*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	–	–	1000	–	–	–
Pyrithiobac (μM)	140	<0.001	–	–	250	–	–	–
Trifluralin (μM)	1	1	–	–	–	–	–	–

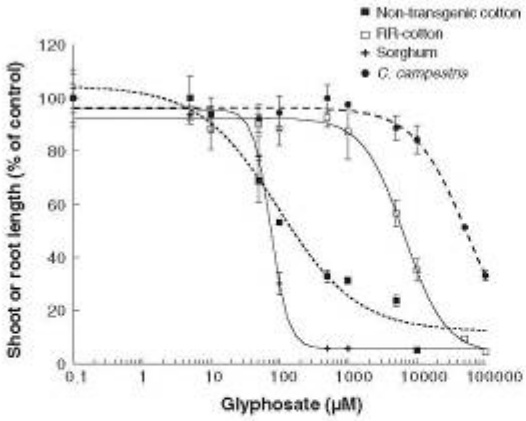

Fig. 1 Effect of glyphosate on the elongation of *Cuscuta campestris* shoots, and sorghum and cotton roots. The assay was conducted in sand-filled Petri dishes. Vertical bars represent SEM.

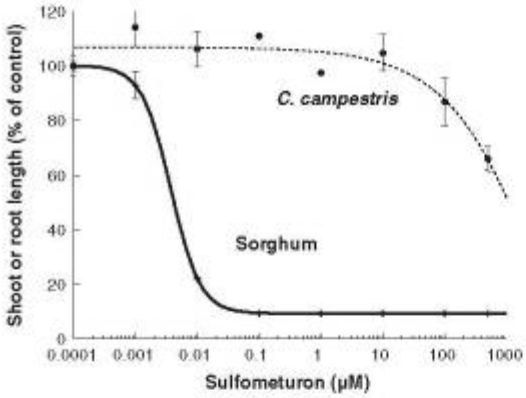

Fig. 2 Effect of sulfometuron on the elongation of *Cuscuta campestris* shoots (●) and sorghum roots (+). Vertical bars represent SEM.

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

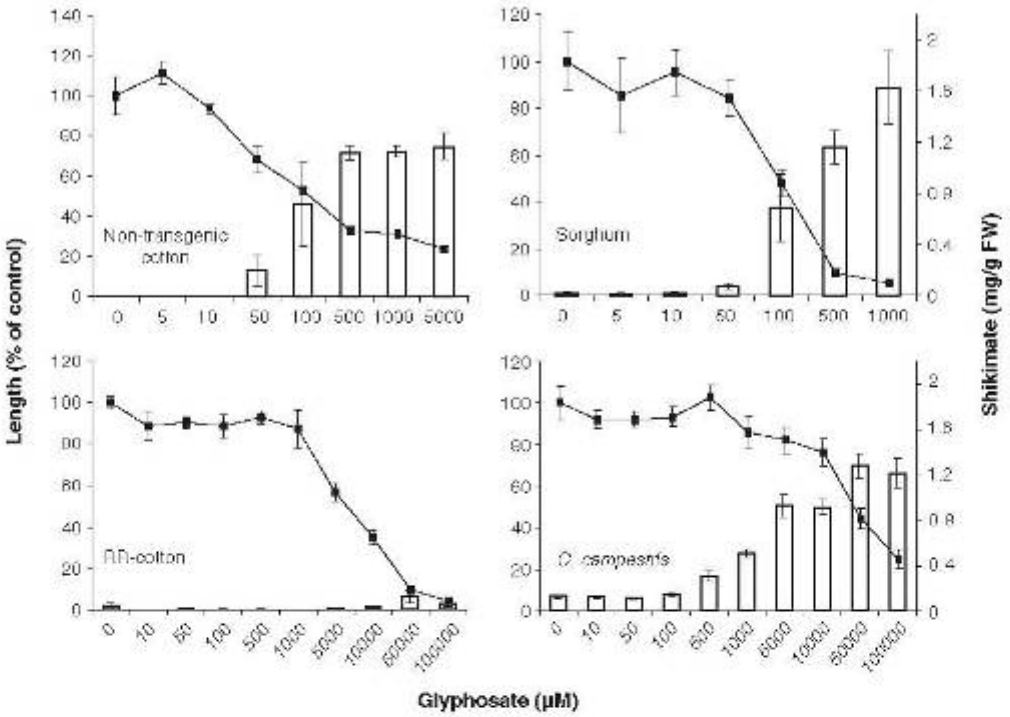

Fig. 3 Shikimic acid accumulation (bar) and root (cotton and sorghum) or shoot (*Cuscuta campestris*) length (line) at different glyphosate concentrations (µM). Vertical bars represent SEM.

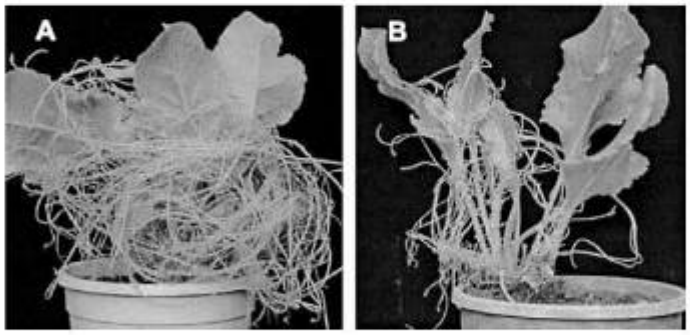

Fig. 4 RR-sugarbeet infested with *Cuscuta campestris* 30 days after treatment, untreated (A) and glyphosate-treated (1.08 kg a.e. ha^{-1}) (B).

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}).

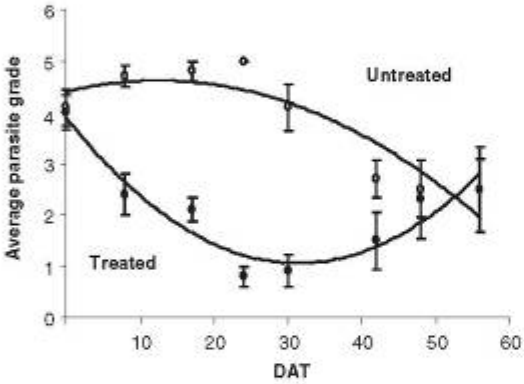

Fig. 5 *Cuscuta campestris* growth [(○) untreated; (●) treated] on RR-sugarbeet plants following post-emergence treatment with glyphosate (0.72 kg a.e. ha^{-1}). Parasite growth was visually evaluated and scored, with 0 representing no parasite growth, and 5 representing full parasite vigour and host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

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References

Amerhein N, Deus B, Gehrke P & Steinrucken HC (1980) The site of the inhibition of the shikimate pathway by glyphosate II. Interference of glyphosate with chorismate formation *in vivo* and *in vitro*. *Plant Physiology* **66**, 830–834.

Bestman HD, Devine MD & Vanden Born WH (1990) Herbicide chlorsulfuron decreases assimilate transport out of treated leaves of field pennycress (*Thlaspi arvense* L.) seedlings. *Plant Physiology* **93**, 1441–1448.

Bewick TA, Binning LK & Balke NE (1991) Absorption and translocation of glyphosate by carrot infected by swamp dodder. *Journal of the American Society of Horticultural Science* **116**, 1035–1039.

Cromartie TH & Polge ND (2000) An improved assay for shikimic acid and its use as a monitor for the activity of sulfosate. *Weed Science Society of America Abstracts* **40,** 291.

Dawson JH, Musselman LJ, Wolswinkel P & Dorr I (1994) Biology and control of *Cuscuta*. *Review Weed Science* **6,** 265 317.

Dorr I (1987) The haustorium of *Cuscuta* new structural results. In: *Proceedings 1987 4th International Symposium on Parasitic Flowering Plants* (eds HC Weber & W Forstreuter), Marburg, Germany, 163 173.

Fer A (1984) Physiological approach to the chemical control of *Cuscuta*: experiments with ^{14}C-labelled herbicide. In: *Proceedings 1984 3rd International Symposium on Parasitic Weeds* (eds C Parker, LJ Musselman, RM Polhill & AK Wilson), Aleppo, Syria, 164 174.

Geiger DR & Bestman HD (1990) Self-limitation of herbicide mobility by phytotoxic action. *Weed Science* **38,** 324 329.

Geiger DR, Shieh WJ & Fuchs MA (1999) Causes of self-limited translocation of glyphosate in *Beta vulgaris* plants. *Pesticide Biochemistry and Physiology* **45,** 124 133.

Hall LM & Devine MD (1993) Chlorsulfuron inhibition of phloem translocation in chlorsulfuron-resistant and susceptible *Arabidopsis thaliana*. *Pesticide Biochemistry and Physiology* **45,** 81 90.

Holm L, Doll J, Holm E, Panch J & Harberger J (1997) *World Weeds: Natural Histories and Distribution*. John Wiley & Sons, NY, USA.

Hutcheson JM & Ashton FM (1980) Germination of field dodder (*Cuscuta campestris*). *Weed Science* **28,** 330 333.

Joel DM, Kleifeld Y, Losner-Goshen D, Herzlinger G & Gressel Y (1995) Transgenic crops against parasites. *Nature* **374,** 220 221.

Kanamitu FK, Ransom JK & Gressel J (2001) Imazapyr seed dressings for *Striga* control on acetolactate synthase target-site resistant maize. *Crop Protection* **20,** 885 895.

Kim S & Vanden Born WH (1996) Chlorsulfuron decreases both assimilate export by source leaves and import by sink leaves in Canola (*Brassica napus* L.) seedlings. *Pesticide Biochemistry and Physiology* **56,** 141 148.

Liu ZQ & Fer A (1990) Effect of a parasite (*Cuscuta lupuliformis* Krock.) on the redistribution of two systemic herbicides applied on a legume (*Phaseolus aureus* Roxb.). *Comptes Rendus de L'Academie des Sciences, Series 3, Science de la Vie* **310,** 333 339.

Liu ZQ, Agaev R, Lecocq M, Fer A & Hallet JN (1987) Effects and mode of action of several herbicides on dodder seedlings (*Cuscuta lupuliformis* Krock). In: *Proceedings 1987 4th International Symposium on Parasitic Flowering Plants* (eds HC Weber & W Forstreuter), Marburg, Germany, 511 521.

Meissner R, Jacobson Y, Melamed S *et al.* (1997) A new model system for tomato genetics. *The Plant Journal* **12,** 1465 1472.

Nir E, Rubin B & Zharasov SW (1996) On the biology and selective control of field dodder (*Cuscuta campestris*). In: *Advances in Parasitic Plant Research. Sixth International Parasitic Weed Symposium* (eds MT Moreno, JI Cubero, D Berner, D Joel, LJ Musselman & C Parker), Cordoba, Spain, 809 816.

Parker C & Riches CR (1993) *Cuscuta* species, the dodders; and *Cassytha Filformis*. In: *Parasitic Weeds of the World: Biology and Control* (eds C Parker & CR Riches). 183 223. CAB International, Wallingford, UK.

Press MC, Graves GR & Stewart GR (1990) Physiology of the interaction of angiosperm parasites and their higher plant hosts. *Plant Cell and Environment* **13,** 91 104.

Ray TB (1984) Site of action of chlorsulfuron. Inhibition of valine and isoleucine biosynthesis in plants. *Plant Physiology* **75,** 827 831.

Seefeldt SS, Jensen JE & Fuerst EP (1995) Log-logistic analysis of herbicide dose response relationships. *Weed Technology* **9,** 218 227.

Sibony M & Rubin B (2003) Molecular basis for multiple resistance to acetolactate synthase-inhibiting herbicides and atrazine in *Amaranthus blitoides* (prostrate pigweed). *Planta* **216,** 1022 1027.

Singh BK & Shaner DL (1998) Rapid determination of glyphosate injury to plants and identification of glyphosate-resistant plants. *Weed Technology* **12,** 527 530.

Tal A, Kotoula-Syka E & Rubin B (2000) Seed-bioassay to detect grass weeds resistant to acetyl coenzyme A carboxylase inhibiting herbicides. *Crop Protection* **19,** 467 472.

Tsivion Y (1979) *The regulation of the association of the parasitic plant* Cuscuta campestris *with its hosts*. PhD thesis, The Hebrew University of Jerusalem, Jerusalem, Israel.

Werner M, Uehlein N, Proksch P & Kaldenhoff R (2001) Characterization of two tomato aquaporins and expression during the incompatible interaction of tomato with the plant parasite *Cuscuta reflexa*. *Planta* **213,** 550 555.

Wolswinkel P (1984) Phloem unloading and 'sink strength': the parallel between the site of attachment of *Cuscuta* and developing legume seeds. *Plant Growth Regulation* **2,** 309 317.

Wolswinkel P, Ammerlaan A & Henricus FCP (1984) Phloem unloading of amino acids at the site of attachment of *Cuscuta europaea*. *Plant Physiology* **75,** 13 20.

Yuan CI, Chaing MY & Chen YM (2002) Triple mechanisms of glyphosate-resistance in naturally occurring glyphosate-resistant plant *Dicliptera chinensis*. *Plant Science* **163,** 543 554.

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ORIGINAL ARTICLE

Talia Nadler-Hassar · Alexander Goldshmidt
Baruch Rubin · Shmuel Wolf

Glyphosate inhibits the translocation of green fluorescent protein and sucrose from a transgenic tobacco host to *Cuscuta campestris* Yunk.

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Abstract The parasitic plant *Cuscuta campestris* is dependent on its host for water, assimilates and amino acids. It can be controlled by the herbicide glyphosate, which inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), resulting in shikimate accumulation. In this study, *C. campestris* was parasitic on transgenic tobacco plants expressing green fluorescent protein (GFP) in the phloem. Changes in [^{14}C]sucrose and GFP accumulation in the parasite were used as indicators of the herbicide's effect on translocation between the host and parasite. Host plants were treated with glyphosate 22 days after sowing. Shikimate accumulation in the parasite 1 day after glyphosate treatment (DAGT) confirmed EPSPS inhibition in *C. campestris*. No damage was visible in the host plants for the first 3 DAGT, while during that same time, a significant reduction in [^{14}C]sucrose and GFP accumulation was observed in the parasite. Thus, we propose that the parallel reduction in GFP and sucrose accumulation in *C. campestris* is a result of a glyphosate effect on the parasite's ability to withdraw assimilates from the host.

Keywords *Cuscuta* · 5-Enolpyruvylshikimate-3-phosphate synthase · Green fluorescent protein · Glyphosate · Sucrose translocation (inhibition)

Abbreviations CLSM: Confocal laser-scanning microscope · DAGT: Days after glyphosate treatment · DAS: Days after sowing · EPSPS: 5-Enolpyruvylshikimate-3-phosphate synthase · GFP: Green fluorescent protein

T. Nadler-Hassar · A. Goldshmidt · B. Rubin (✉) · S. Wolf
R.H. Smith Institute of Plant Sciences and Genetics in Agriculture, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem,
76100 Rehovot, Israel
E-mail: rubin@agri.huji.ac.il
Tel.: +972-8-9489248
Fax: +972-8-9362083

Introduction

Cuscuta campestris (field dodder), a member of the Convolvulaceae family, is a nonspecific parasite, capable of attacking (sometimes simultaneously) a broad range of host species. This, and its wide geographical distribution, makes it a serious problem weed for many crops and vegetables and among the most damaging parasites worldwide (Parker and Riches 1993; Holm et al. 1997). The germinating seed produces a long, thin, rootless, yellow-orange leafless stem that coils around the host stem. 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 (Press et al. 1990). After attachment, the parasite penetrates the host tissue and develops 'searching hyphae' from the epidermal cells of the haustorium. These hyphae elongate within the host tissue in search of its xylem and phloem (Vaughn 2003). A highly efficient connection allows the parasite to divert resources (water, assimilates and amino acids) from the host to itself (see review by Dawson et al. 1994). Wolswinkel (1984) describes the remarkable ability of *C. campestris* to attract resources from the host, as a "super-sink" that competes with the developing fruits for assimilates.

Inhibitors of amino acid biosynthesis, such as glyphosate, have been found effective at controlling *C. campestris* (Liu and Fer 1990; Bewick et al. 1991; Dawson et al. 1994; Nir et al. 1996). The herbicide glyphosate interrupts the biosynthesis of aromatic amino acids (phenylalanine, tyrosine and tryptophan) in plants by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimate pathway. Inhibition of this pathway leads to the overproduction and accumulation of shikimate

(Amrhein et al. 1980; Geiger and Bestman 1990). The uncontrolled flow of carbon to the shikimate pathway and the lack of essential amino acids disrupt additional pathways (Devine et al. 1993). Several studies have indicated that glyphosate's growth-inhibitory effects on higher plants can be alleviated by the addition of phenylalanine (Jaworski 1972), or a combination of phenylalanine and tyrosine (Gresshoff 1979) to the growth medium. The exact cause of the plant's death after glyphosate application is not yet clear, but there is evidence that besides inhibiting EPSPS, glyphosate may also inhibit the translocation of assimilates from source leaves to various sinks (Geiger and Bestman 1990; Geiger et al. 1999).

Earlier studies established that glyphosate is accumulated mainly in the parasite after application of the herbicide to a *C. campestris*-parasitized host plant (with no tolerance to glyphosate) due to the former's strong sink activity. As a result, parasite growth is inhibited with no significant damage to the host (Fer 1984; Liu and Fer 1990; Bewick et al. 1991; Dawson et al. 1994; Nir et al. 1996). Moreover, in a recent study we were able to show that when the parasite is grown independently of a host, it can tolerate high concentrations of glyphosate. In addition, application of glyphosate to a parasitized glyphosate-resistant sugar beet host initially injured the parasite, but at a later stage the parasite recovered and resumed normal growth (Nadler-Hassar and Rubin 2003). Collectively, these results indicate that the parasite has some natural mechanism of glyphosate tolerance. A possible mechanism could relate to the ability of the parasite to withdraw amino acids from the host while its own biosynthetic pathway is disrupted. However, this should only occur while the parasite is attached to a host. Hence, inhibition of amino acid biosynthesis may not be lethal for the parasite, and inhibition of EPSPS may not be the sole mechanism by which glyphosate affects *C. campestris* development.

Our hypothesis is that *C. campestris* is injured by the herbicide mainly because of the inhibited translocation of assimilates from the host, rather than the direct effect of the herbicide on amino acid biosynthesis in the parasite. In the present study we developed a unique experimental system that allowed a direct characterization of the kinetics of protein and sucrose transport from a host plant to *C. campestris*. Tobacco plants expressing a green fluorescent protein (GFP) in the sieve element companion cell complex, under the control of the *Arabidopsis thaliana* sucrose transporter (AtSUC2) promoter (Imlau et al. 1999; Oparka et al. 1999), were employed to monitor the effect of glyphosate on the traffic of the fluorescent protein to the parasite. The influence of glyphosate on carbon transport in the phloem was based on pulse chase experiments after the application of [^{14}C]sucrose to the source leaves. We demonstrate that, following glyphosate application, phloem transport to the parasite is inhibited before detectable changes in the biosynthesis of aromatic amino acids can be measured.

Materials and methods

Plant material

One-month-old transgenic *Nicotiana tabacum* L. cv. Samsun seedlings expressing GFP in their companion cells (*At*SUC2 GFP) kindly provided by Prof. N Sauer, (Universität Erlanger-Nürnberg, Erlangen, Germany), were transferred to pots (9 cm × 9 cm × 9.5 cm) containing a mixture of peat and 1 g kg^{-1} of slow-release fertilizer (Osmocote 14-14-14; Grace Sierra International, Heerlen, The Netherlands). Three days later, 25 acid-scarified seeds of *Cuscuta campestris* Yunk., collected in Or Haner, Israel (1994), were sown next to each tobacco plant. Pots were placed in a heated greenhouse with an average temperature of 26/16°C (day/night).

Glyphosate treatment

The effect of glyphosate [Roundup, 360 g Acid Equivalent (a.e.) l^{-1}; Monsanto, St. Louis, MO, USA] on the movement and accumulation of GFP and sucrose in the parasite's tissue was tested with tobacco plants at the six- to seven-leaf stage. Glyphosate (36 μg a.e.) was applied to the third leaf (10 1-μl drops) where leaf 1 was defined as the youngest to reach 3 cm in length. *C. campestris* and host plant tissue were sampled for confocal laser-scanning microscopy (CLSM) examination, shikimic acid levels, and protein extractions for immune blots, 3, 7, 10 and 14 days after glyphosate treatment (DAGT).

Confocal laser-scanning microscopy

The pattern of GFP accumulation in the parasite and host was determined by evaluating fluorescence in the tissue at 14, 22, 25, 29, 32 and 36 days after sowing (DAS) the parasite and at 3, 7, 10, and 14 DAGT. Samples were examined by CLSM (model LSM510; Zeiss, Jena, Germany) using blue laser (25 mW argon) excitation light (488 nm). GFP expression in the main vein cells of tobacco leaves was assessed on transverse sections (200 μm thick) using a vibratome (Series 1000 Sectioning System; Vibratome, St. Louis, MO, USA). *C. campestris* shoots were cut longitudinally prior to viewing.

GFP detection-gel blots

Frozen *C. campestris* (1:3, w/w) and host tissue (1:4, w/w) were homogenized in extraction buffer [9 M urea, 75 mM Tris buffer (pH 6.8), 4.5%, v/v, SDS and 7.5%, v/v, β-mercaptoethanol]. After grinding, samples were left at room temperature for 30 min, boiled for 5 min and then centrifuged at 10,000 *g* for 10 min. The supernatant was brought to a final concentration of 10% (v/v)

glycerin. Protein concentrations of all samples were compared by the NIH Image/FAT 1.6 on scanned pictures of Coomassie-stained SDS polyacrylamide gels (Sambrook et al. 1989). Equal amounts of protein were separated on SDS polyacrylamide gels and transferred to nitrocellulose filters. GFP was detected with a polyclonal anti-GFP antiserum (1:500; Clontech, Palo Alto, CA, USA).

Monitoring the effect of glyphosate on sucrose movement

C. campestris-infested tobacco plants were transferred from the greenhouse to a growth chamber (12 h light at 24°C and 18°C dark). Glyphosate (36 µg a.e.) was applied to one half of the host leaf and 24 h later [^{14}C]sucrose (15,000 Bq, specific activity 6.29×10^{7} Bq mg^{-1}; Amersham Pharmacia Biotech) was applied to the other half of the leaf (10 1-µl drops), 9 10 replications per treatment. The treated host leaf, the host apex and the parasite were separated from the host 30, 36, and 48 h after herbicide application. All plant parts were weighed and freeze-dried before combustion in a sample oxidizer (Model 307; Packard, Downers Grove, IL, USA). ^{14}C content in each sample was counted in a liquid scintillation analyzer (Tri-Carb 1600TR; Packard) and the results of the assay are presented as Bq per g fresh weight (FW).

Shikimic acid detection

Plant tissue was tested for shikimate content using the method of Singh and Shaner (1998) with modifications made by Cromartie and Polge (2000).

Statistical analysis

All experiments were performed in a completely randomized design with at least five replications. Means and standard errors were calculated for all assays.

Results

Time course of GFP movement from host to parasite

Parasite seedlings established their haustoria in the host tissue and began to develop a new apex 13 14 DAS. Untreated parasitized host plants continued to develop normally until 25 DAS, when they began to show stress symptoms due to the heavy infestation of the parasite. The decline in host growth was followed by a gradual decline in parasite growth up to 40 DAS, when all hosts and *C. campestris* plants died (Fig. 1).

The time course of GFP movement from the host to the parasite was determined by the accumulation of fluorescence, as detected by CLSM and immune blots with GFP antibodies specific to parasite protein extractions. Early fluorescent signals were detectable by CLSM in the *C. campestris* apex 14 DAS, and reached their highest level at 25 DAS (Fig. 2). The accumulation of fluorescence in the parasite was followed by a steady decline that paralleled the gradual decline in the vitality of the host, which was heavily infested with *C. campestris* (Figs. 1, 2). Immune blots with GFP antibodies specific to parasite protein extractions supported the observations made by CLSM. Until 25 DAS, GFP accumulated in the parasite, after which there was a steady decline that corresponded with the signs of host stress, indicating that the GFP accumulation pattern in the parasite (Fig. 3) is related to host collapse.

The effect of glyphosate on the parasite

Based on the established time course, glyphosate was applied to the parasitized host 22 DAS. Parasite growth and development ceased 3 DAGT; however, during the next few days, some of the parasite shoots died while other shoots turned green and began to develop new, very compact and deformed shoots from which, at a later stage, a new developing apex emerged. Unexpectedly, all of the glyphosate-treated host plants and some of the parasite shoots were still alive 18 DAGT (40 DAS), in contrast to the untreated host plants which, by the end of the assay, were severely damaged or dead (Fig. 1).

The effect of glyphosate on GFP accumulation in the parasite

The changes in GFP level in the parasite following glyphosate treatment were evaluated to assess the effect of the herbicide on translocation between the host and *C. campestris*. At 3 DAGT, there was a significant reduction in GFP levels in the parasite as compared to the increasing GFP levels seen in *C. campestris* growing on untreated hosts (Fig. 3, 22 vs. 25 DAS). At 7 DAGT, the GFP in *C. campestris* growing on herbicide-treated hosts was undetectable with the antibody, while the GFP level in *C. campestris* growing on untreated hosts was slightly lower (at 29 DAS) than that detected 25 DAS (Fig. 3).

A similar reduction in fluorescence in *C. campestris* growing on herbicide-treated hosts was visible at 7 DAGT, and fluorescence continued to decline until it became undetectable at around 14 DAGT (Fig. 2). *C. campestris* infestation and glyphosate did not reduce fluorescence levels in young host tobacco leaves (Fig. 4).

The effect of glyphosate on sucrose accumulation in the parasite

Monitoring changes in the accumulation of [^{14}C]sucrose (applied 24 h after glyphosate treatment) in the parasite after glyphosate application to the host offers an alter-

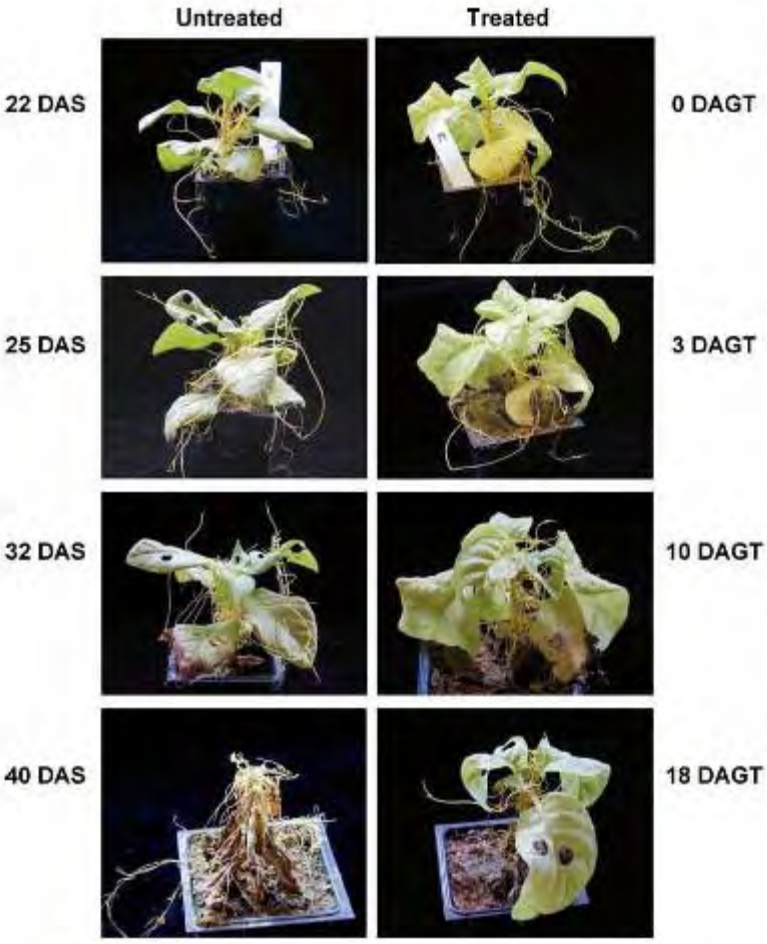

Fig. 1 *Cuscuta campestris* development on untreated (days after sowing—DAS) and treated (days after glyphosate treatment—DAGT) tobacco (*Nicotiana tabacum*) hosts. Glyphosate (36 μg a.e. plant^{-1}) was applied to the host leaves 22 DAS

native method of assessing the influence of the herbicide on assimilate translocation from the host to the parasite. The changes in [^{14}C]sucrose accumulation correlated with those seen in GFP accumulation. Forty-eight hours after glyphosate application, the [^{14}C]sucrose in *C. campestris* was reduced by 50% as compared to that in *C. campestris* attached to untreated hosts (Fig. 5).

Shikimate detection

The accumulation of shikimic acid is an indication of EPSPS inhibition by glyphosate (Amrhein et al. 1980; Geiger and Bestman 1990). Shikimate was detectable in the parasite as early as 1 DAGT, confirming that the herbicide had rapidly reached its target site. Shikimate accumulated continuously over time in the parasite, but not in the host (Fig. 6). Three DAGT, the apex of nonparasitized hosts had accumulated high shikimate levels; in contrast, very little shikimate had accumulated in the apex of glyphosate-treated hosts parasitized with *C. campestris* (Fig. 7).

Discussion

The presented results indicate that glyphosate treatment inhibits the translocation of proteins and sucrose from tobacco plants to the parasite *C. campestris*. The small number of plasmodesmata interconnecting the hyphae of *Cuscuta* and the sieve element–companion cell complex of the host has led to the hypothesis that assimilate movement between the host and the parasite is via the apoplast (Wolswinkel 1986; Dawson et al. 1994; Jeschke et al. 1994). Extended movement of GFP from transgenic tobacco plants expressing AtSUC2–GFP to the parasite *C. reflexa* was observed at 14–16 days after attachment of the parasite (Haupt et al. 2001). The accumulation of GFP in *C. campestris* at 14–25 DAS in our study (Fig. 2) further supports the hypothesis that a symplasmic pathway is also involved in the transport of assimilates from the host to the parasite. Moreover, those results indicate that in addition to solutes, the parasite can withdraw macromolecules from the host, including proteins.

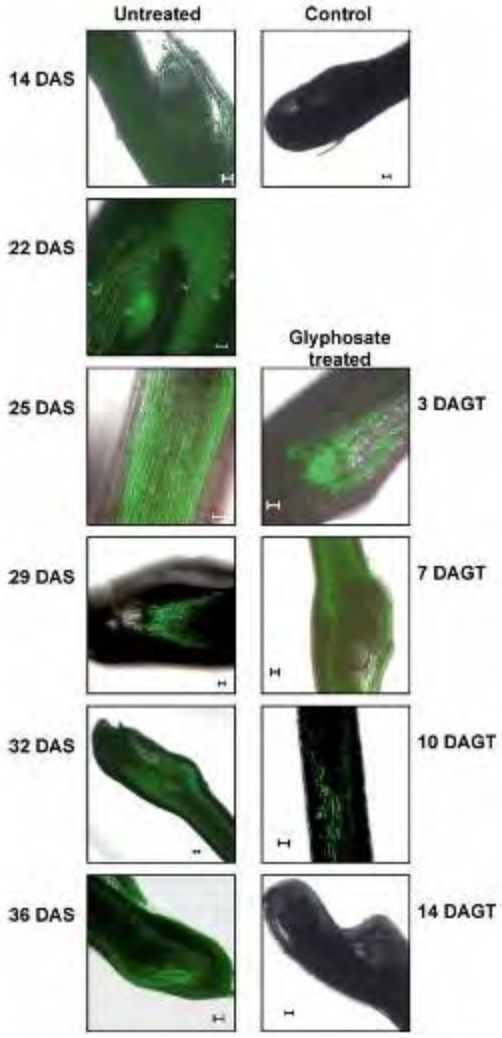

Fig. 2 Green fluorescent protein (GFP) accumulation in *C. campestris* shoots growing on untreated tobacco plants 14–36 DAS; at 22 DAS, the host was treated with glyphosate (36 μg a.e. plant^{-1}) and GFP was monitored in the shoots 3–14 days after glyphosate treatment (DAGT). Control is a *C. campestris* shoot on a nontransgenic tobacco host. Bar = 50 μm

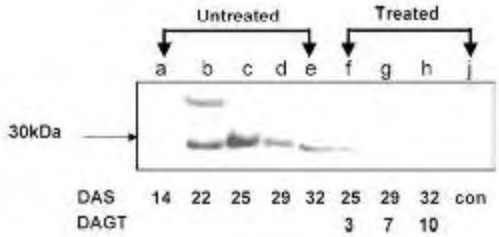

Fig. 3 Quantitative immunoblots of GFP in total protein extracts (equal amounts of protein were loaded in each lane) of *C. campestris* growing on untreated and glyphosate (36 μg a.e. plant^{-1})-treated hosts (22 DAS). The control (*con*) was taken from *C. campestris* growing on nontransgenic tobacco

The reduction in GFP levels in the parasite starting at 25 DAS coincided with symptoms of damage observed on the host. This damage was caused by the long and heavy infestation of the parasite that deprived the host of nutrients and assimilates and interfered with its normal metabolism and development (Press et al. 1990). These results indicate that GFP movement into the parasite can serve as an indicator of the condition of the parasite, the host, and/or their interaction.

One of the most important findings in our study was the rapid effect of glyphosate application on GFP accumulation in *C. campestris*. A significant reduction in GFP accumulation in the parasite was observed within 3 DAGT. Interestingly, a concomitant reduction was observed in the accumulation of [^{14}C]sucrose in the parasite. This reduction could be due to the inhibition of metabolic activity within the host (carbon and nitrogen metabolism), or to a direct effect of glyphosate on assimilate translocation from the host to the parasite.

No visible injuries were observed in the host plants 3 DAGT. Moreover, a quantitative analysis of GFP levels in the source leaves of the host tobacco plants indicated no changes in GFP production by the latter. These observations suggest that the parallel reduction in GFP and [^{14}C]sucrose accumulation in *C. campestris* 3 DAGT was not a result of glyphosate's influence over the host metabolism but, rather, the result of a weakening effect on the parasite's ability to act as a sink and withdraw assimilates from the host.

A possible explanation for the influence of glyphosate on the process of phloem transport from the host to the parasite may relate to the rapid accumulation of the herbicide in the parasite. This is supported by the analyses of shikimate levels in both the host sinks and the parasite. Shikimate accumulation in glyphosate-treated plants is the direct result of EPSPS inhibition by the herbicide (Amrhein et al. 1980; Geiger and Bestman 1990). The high shikimate levels detected in the parasite 1 DAGT confirmed the latter's uptake of glyphosate and the inhibition of EPSPS activity. The significant increase in shikimate levels was observed only in the parasite, and not in the apex (sink) or other untreated leaves of the host, indicating the strong and nonselective sink capacity of the parasite to draw most of the herbicide applied to the host during the first hours after application. This conclusion is supported by our previous study (Nadler-Hassar and Rubin 2003), which demonstrated shikimate accumulation in glyphosate-treated *C. campestris* growing without a host plant. The rapid movement and accumulation of the herbicide in the parasite may protect the host by reducing its herbicide concentrations to ineffective levels (Bewick et al. 1991; Dawson et al. 1994; Nir et al. 1996). This would explain why the treated host plants were still alive 18 DAGT.

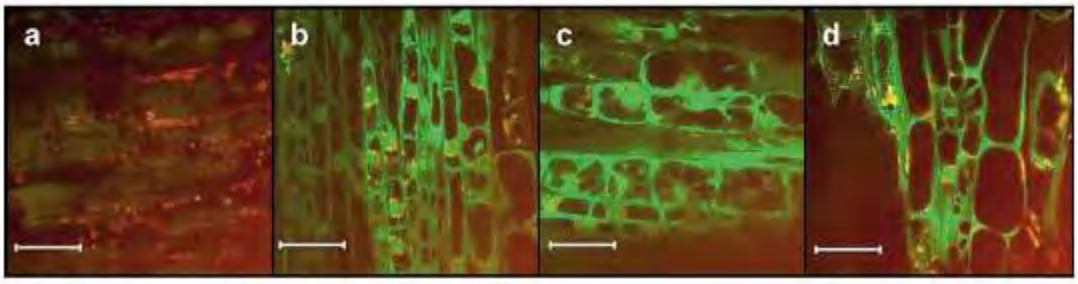

Fig. 4a–d Longitudinal sections of young tobacco leaves 36 days after sowing *C. campestris*. **a** Nontransgenic tobacco, *C. campestris*-infested host. **b** Transgenic tobacco not infested with *C. campestris*. **c** Transgenic tobacco infested with *C. campestris*. **d** Glyphosate-treated transgenic tobacco infested with *C. campestris* 14 DAGT. Bars = 50 μm

The reduction in [^{14}C]sucrose and GFP accumulation following glyphosate application supports our hypothesis that the parasite's growth is inhibited, at least in part, by assimilate starvation rather than by direct herbicide inhibition of the parasite's EPSPS. Although glyphosate inhibits the EPSPS of *C. campestris* attached to a resistant host or, in this case, to an undamaged host, the parasite has an alternative source from which to draw the needed amino acids (our unpublished data; Fer 1981; Wolswinkel et al. 1984). Therefore, a continuous normal rate of amino acid transport from the host to the parasite should alleviate the inhibition of amino acid biosynthesis in the parasite after glyphosate treatment (Jaworski 1972). Alternatively, the effect of glyphosate on the EPSPS of *C. campestris* may be accompanied by additional injury caused to the parasite. Such injury could explain the reduction (or loss) of the parasite sink strength, resulting in the inhibition of translocation to the parasite. On the other hand, one cannot rule out the possibility that the herbicide does have a sub-lethal effect on the host, which causes an alteration in the allocation of assimilate and reduced translocation to the parasite.

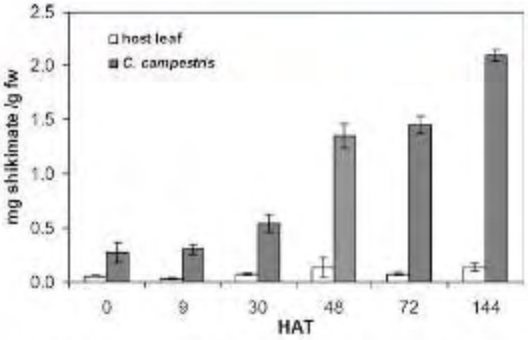

Fig. 6 Shikimate accumulation in glyphosate-treated *C. campestris* and tobacco host leaf (three replicates). *HAT* Hours after treatment. Means ± SE

The research presented here indicates that starvation of *C. campestris* due to the inhibition of photoassimilate translocation from the host is a major cause of glyphosate's ability to control the parasite. Further study is being aimed at dissecting the specific site(s) of the herbicide's action in the process of phloem transport from the host to the parasite. The parasite–host model used in this study can be used in the future to further delineate the mechanisms by which glyphosate or other amino acid biosynthesis inhibitors affect treated plants.

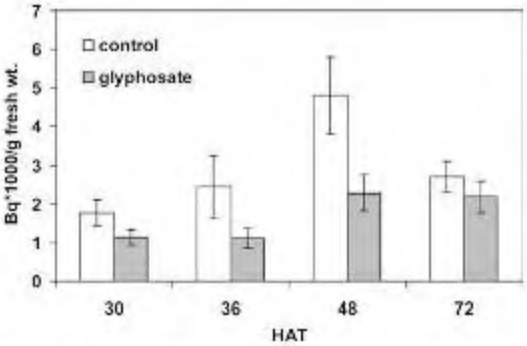

Fig. 5 [^{14}C]Sucrose accumulation in *C. campestris* growing on untreated and glyphosate-treated transgenic tobacco, 30, 36, 48 and 72 h after treatment (*HAT*). Means ± SE

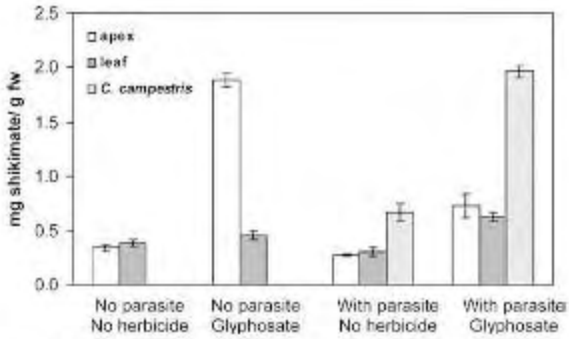

Fig. 7 Shikimate accumulation in *C. campestris*, and treated host leaf and apex 72 h after glyphosate treatment (five replicates). Means ± SE

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References

Amrhein N, Deus B, Gehrke P, Steinrucken HC (1980) The site of the inhibition of the shikimate pathway by glyphosate. II. Interference of glyphosate with chorismate formation in vivo and in vitro. Plant Physiol 66:830 834

Bewick TA, Binning LK, Balke NE (1991) Absorption and translocation of glyphosate by carrot infected by swamp dodder. J Am Soc Hort Sci 116:1035 1039

Cromartie TH, Polge ND (2000) An improved assay for shikimic acid and its use as a monitor for the activity of sulfosate. In: Abstracts of the proceedings of the annual conference of the Weed Science Society of America, vol 40, #291

Dawson JH, Musselman LJ, Wolswinkel P, Dorr I (1994) Biology and control of *Cuscuta*. Rev Weed Sci 6:265 317

Devine MD, Duke SO, Fedtke C, eds (1993) Inhibition of amino acid biosynthesis. In: Physiology of herbicide action. Prentice-Hall, Inglewood Cliffs, NJ, pp 251 294

Fer A (1981) Transport pathways involved in nutrient supply of a phanerogamous parasitic plant. Study with detached leaves parasitized by *Cuscuta*. Physiol Veg 19:177 196

Fer A (1984) Physiological approach to the chemical control of *Cuscuta*: experiments with ^{14}C-labeled herbicides. Proceedings of the 3rd international symposium on parasitic weeds, Aleppo, pp 164 174

Geiger DR, Bestman HD (1990) Self-limitation of herbicide mobility by phytotoxic action. Weed Sci 38:324 329

Geiger DR, Shieh WJ, Fuchs MA (1999) Causes of self-limited translocation of glyphosate in *Beta vulgaris* plants. Pestic Biochem Physiol 45:124 133

Gresshoff PM (1979) Growth inhibition of *Arabidopsis thaliana* by glyphosate and its reversal by phenylalanine and tyrosine. Aust J Plant Physiol 6:177 185

Haupt S, Oparka KJ, Sauer N, Neumann S (2001) Macromolecular trafficking between *Nicotiana tabacum* and the holoparasite *Cuscuta reflexa*. J Exp Bot 52:173 177

Holm L, Doll J, Holm E, Pancho J, Herberger J (1997) In: World weeds: natural histories and distribution. Wiley, New York, pp 249 264

Imlau A, Truernit E, Sauer N (1999) Cell-to-cell and long-distance trafficking of the green fluorescent protein in phloem and symplastic unloading of the protein into sink tissues. Plant Cell 11:309 322

Jaworski EG (1972) Mode of action of N-phosphonomethylglycine: inhibition of aromatic amino acid biosynthesis. J Agric Food Chem 20:1195 1205

Jescke WD, Baumel P, Rath N, Czygan F-C, Proksch P (1994) Modeling of the flows and partitioning of carbon and nitrogen in the holoparasite *Cuscuta reflexa* Roxb. and its host *Lupinus albus* L. II. Flows between the host and parasite and within the parasitized host. J Exp Bot 45:801 812

Liu ZQ, Fer A (1990) Effect of a parasite (*Cuscuta lupuliformis* Krock.) on the redistribution of two systemic herbicides applied on a legume (*Phaseolus aureus* Roxb.). C R Acad Sci Ser 3, Sci Vie 310:333 339

Nadler-Hassar T, Rubin B (2003) Natural tolerance of *Cuscuta campestris* Yunk. to herbicides inhibiting amino acid biosynthesis. Weed Res 43:341 347

Nir E, Rubin B, Zharasov SW (1996) On the biology and selective control of field dodder (*Cuscuta campestris*). In: Moreno MT, Cubero JI, Berner D, Joel D, Musselman LJ, Parker C (eds) Advances in parasitic plant research. Sixth international parasitic weed symposium, Cordoba, pp 809 816

Oparka KJ, Roberts AG, Boevink P, Santa Cruz S, Roberts I, Pradel KS, Imlau A, Kotlizky G, Sauer N, Epel B (1999) Simple, but not branched, plasmodesmata allow the nonspecific trafficking of proteins in developing tobacco leaves. Cell 97:743 754

Parker C, Riches CR, eds (1993) Parasitic weeds of the world: biology and control. CAB International, Wallingford, UK, pp 183 223

Press MC, Graves JD, Stewart GR (1990) Physiology of the interaction of angiosperm parasites and their higher plant hosts. Plant Cell Environ 13:91 104

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Singh BK, Shaner DL (1998) Rapid determination of glyphosate injury to plants and identification of glyphosate-resistant plants. Weed Technol 12:527 530

Vaughn KC (2003) Dodder hyphae invade the host: a structural and immunocytochemical characterization. Protoplasma 220:201 207

Wolswinkel P (1984) Recent development in phloem transport research. The parallel between the site of attachment of *Cuscuta* and developing seeds of legumes. Proceedings of the 3rd international symposium on parasitic weeds, Aleppo, pp 156 163

Wolswinkel P, Ammerlaan A, Henricus FCP (1984) Phloem unloading of amino acids at the site of attachment of *Cuscuta europaea*. Plant Physiol 75:13 20

Wolswinkel P (1986) Phloem unloading in stem parts parasitized by *Cuscuta*. In: Cronshow J, Lucas WJ, Giaquinta RT (eds) Phloem transport. Liss, New York, pp 239 246

Appendix III.

The report on my training at the Hebrew University of Jerusalem (faculty of agriculture, Rehovot) - November 10 to December 26, 2004.

My training at the Hebrew University of Jerusalem (Faculty of Agriculture, Rehovot) passed from November 11 till December 26, 2004.
In the first day of my work Professor Baruch Rubin has acquainted me with the equipment and materials of the laboratory. Many questions have been arisen me about structure of education at the university.
Dr. Rubin has asked me about the Kazakhstan part of the project. On the same day Dr. Rubin has presented me to the employees of his laboratory. Then the Professor has acquainted me with M. Sc. Orly. Orly during all my training period helped and trained me in methods of working on the project.
During the work I have carried out some experiences in laboratories with the purpose of comparison of response assay of field dodders (*Cuscuta campestris*) and *Sorghum* to various concentrations of herbicides.
Experiments performed in Petri dishes filled with thoroughly washed coarse sand (135 g) containing different concentrations of the herbicide solution (15 ml). After sowing the Petri dishes were sealed in a dark room at temperature of 26-28 C^0 at an 80% angle. *Cuscuta campestris* shoots and *Sorghum* roots length were measured at 7 days. The dose-response results of the experiment were plotted on a Logarithmic scale. We used the following herbicides in various concentrations:

1. Kerb, (Pronamide 50%): (0,005 ppm; 0,1 ppm; 0,5 ppm; 1 ppm; 5 ppm; 10 ppm)
2. Raptor (Imazamox,12 %): (0,05 ppm →10 ppm)
3. Pursuit, (Imazethapyr 10 %): (0,05 ppm; 0,1 ppm; 0,5 ppm; 1,0 ppm; 5,0 ppm; 10,0 ppm)
4. Roundup (Glyphosate 36 %): (0,1 ppm; 0,5 ppm; 1 ppm, 5 ppm, 10 ppm, 100 ppm).
5. Flexidor (Isoxaben 50 %): (0,05 ppm → 10 ppm)

Herbicides had different effects on germination and elongation of *Sorghum* roots and *Cuscuta campestris* shoots, but by our data was shown the greatest effect of Isoxaben. *Cuscuta campestris* seeds also were germinated on artificial nutrient medium Murashigi Skug with adding of hormones and antibiotics.
The growth and development of field dodders and its host Canola were determined in greenhouse conditions in 11 cm pots, the substratum has consisted of sandy soil, peat and fertilizers. *Cuscuta campestris* seeds were sown near the host stem after formation of 3-4 leaves of Canola. After 2 weeks, pots were treated with Imazamox.

I have also visited the north of Israel where the big areas of winter wheat are concentrated. I have seen fields with skilled variants of laboratory where in the crop rotation were a soybean, sweet potato, corn, sunflower and fallow. Unfortunately, I could not see other skilled fields of the laboratory, during this period the crop has already been collected. I have conducted the work in greenhouse and laboratory conditions. I had used the digital camera.

At present the skills received in Israel are used by me at the Institute of Plant Protection, Kazakhstan.

In summary, my visit to Israel was very interesting and fruitful. Unfortunately, our conditions do not allow to conduct the experiments at a such high level.

It was my first training, as I already understand now, in this work I have not taken into account many questions and some details, therefore, sometimes industrial experiments with field dodder in our local conditions should be studied in details independently.

I would like to say thanks to Dr. Rubin and his family for wonderful hospitality and realization of all my needs in your country. And also huge gratitude to all employees of the laboratory for their assistance in my work.

For all period of my training I have received 7800 shekels. Also my living in dormitory the professor has undertaken charges. The cost of my air tickets through Istanbul makes $1178

Zhanna Issina,
Kazakh Institute of Plant Protection

Appendix IV: Visit report of the PI to KIPP

Mrs. Elinor Slater,
Coordinator, US-Sponsored Research Programs
Authority for Research and Development
Hebrew University of Jerusalem August 7, 2003
Jerusalem.

Dear Elinor,

Report on my visit to Almaty, Kazakhstan (July 6 to 14, 2003)

Accompanied by my host, Dr. Shaken U. Zharasov I visited the 'Al-Faraby Kazakh National University' which is considered to be the best University in Kazakhstan. We met with Dean of the Faculty of Biology Prof. Dr Bersimbaev Rakhmetkaji and Prof. Tamara M. Shalakhmetova, the Director of the Institute of Ecology. We discussed possibilities to transfer the Dr. Zharasov CDR project to their Faculty and allow him to operate from their facilities. I made it clear that they should take responsibilities for accounting and reporting for all the funds associated with grant activity. They agreed to that and requested that more of their students will be involved in the project. I outlined the academic requirements we ask from a grad students to be associated with the research project.

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.

At the IKPP I figured that the sprayer purchased during the previous CDR project is not operated due to lack of a compressor that was stolen several years ago (the theft was reported by Zharasov to the Police, HUJ, etc.). Because he did not have the money to purchase a new one the whole equipment was not used. I bought a new compressor (US$ 65.0) in Almaty, installed it, fixed the sprayer and re-calibrated it. Now it is working properly and I trained Dr. Zharasov's team, a technician and a research student, how to operate it properly.

I also visited several farms where Dr. Zharasov conducts his field research. The farms are spread apart, approximately one hour drive from the center of Almaty. These mixed farms that grow vegetables, field crops and animals, are all heavily infested with field dodder. Dodder causes severe damage to sugarbeet, red beets, safflower and alfalfa. Due to lack of funds the farmers are not using herbicides to control the dodder although it was clear from the experiments run by Dr. Zharasov on their fields, that wise use of herbicides can solve the problem. Unfortunately, Dr. Zharasov was not able to conduct more field trials or to drive further to the countryside.

My discussions with Dr. Zharasov were conducted with the assistance of Miss Aijan Jusupova, a 4th year student at the 'Al-Faraby Kazakh National University' (biotechnology major), who speaks reasonable English and works with Dr. Zharasov on the project. She was appointed by Dr. Zharasov as a candidate for training in our laboratory. 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. It seems though that Aijan might be a good candidate for training at the HUJ as soon as she graduates her first degree and starts post-graduation studies, which according to Dean Bersimbaev Rakhmetkaji is quite sure.

In summary, the visit was very fruitful, as I have seen, first hand, the difficult situation in which Dr. Zharasov is functioning and better understand the new conditions created by the recent administrative changes.

I suggest providing Dr. Zharasov through the KIPP with in advance funds sufficient to operate the project. I think that $10,000 will cover the inland travel, expendables and salaries for workers both in the lab and field for several months. I also suggest encouraging him to send the KIPP approved Financial Reports more frequently, so funds flow will be facilitated.

I would like to take this opportunity and thank Dr. Zharasov and his family for the wonderful hospitality and for their endless efforts to make my visit a pleasant one and to accommodate my special needs in a foreign country.

CC. Boaz Ayalon, US Embassy