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B) Table of Contents

C) Executive Summary.....3

D) Research Objectives.....4

E) Methods and Results.....5

F) Impact, Relevance, and Technology Transfer.....24

G) Project Activities/Outputs 25

H) Project Productivity 26

I) Future Work.....26

J) Literature Cited 27

C) Executive Summary

The overall objectives of our project were to eliminate legume devastation caused by the parasitic weed broomrape in the Middle East and other parts of the world. Our approach was multi-disciplinary based on close collaboration between the two groups (Israeli and Palestinian) in order to achieve the following objectives: Identify crop resistance to broomrape, refine herbicidal methods for broomrabe control by using low rates of crop selective herbicides, developing agronomic measures and developing biological control methods to broomrape as a specific bioherbicides. Approaches that have shown some degree of success are mainly the use of herbicides and of resistant crops. However, only a limited number of Orobanche resistances are available, for sunflower and few legumes, and very few herbicides can be applied against Orobanche, and these are selective enough only with a limited number of crops.

Overall, excellent progress has been made toward project objectives in the following aspects: Several lines from different crops (faba bean, vetch spp. broad bean, soy bean, cow pea, chick peas, lentil and trifolium spp.) were screened for resistance to O. aegyptiaca and O. crenata. A few lines (Popany, Sadot, 1, 106, Achziv 2 and some lines of soybean, cowpea) demonstrated high resistance toward the parasites, lines that have high level of resistance were exchanged and shared between the two teams for testing under their local conditions. Using low rates of the crop selective herbicides (imazapic, imazethapyr and imazaquin), they significantly reduced the total number of parasites counted on the roots and in some cases prevented completely parasite inflorescences emergence above the soil. In our research project we also focused on biological control approaches mainly isolation of a new pathogen of parasitic plant *Orobanche* spp. Most fungi isolated were identified as *Fusarium* spp. (119 isolates) and *Rhizoctonia solani* (14 isolates). Most effective isolates were tested for their efficacy in Orobanche control in legumes and had been exchanged between the two countries.

Developing agronomic measures such as planting time catch and trap crops to reduce broomrape seed bank in the soil had been also tried. Our results indicate that altering chickpea sowing dates has no practical advantage in preventing O. aegyptiaca and O. crenata damage to the chickpea plants. Pepper as a catch crop could not be effective for winter crops such as legumes, but it could be used and adapted as a trap crop in an integrated control system in the summer season for summer crops. More experiments with a wider variety collection are needed in order to establish this hypothesis.

Despite the political situation prevailed in the area, cooperation has been extremely close during the project period. Initially, the collaborating partners communicated by email to discuss science and coordinate the project, but although, our Co-PI from the Palestinian Authority Dr. Radwan Barakat, has visited Israel several times. These visits were enormously valuable, allowing exchange of ideas and information, and providing a close link with the Newe Ya'ar group, where the Israeli group acquired considerable experience in developing, testing and applying methods for broomrape control. This knowledge and techniques were recently transferred to Palestinian scientists by Dr. Radwan for training and adapting it to Palestinian students and technicians.

Additionally, some resistant or tolerant legume species to broomrape and some effective isolates of bioherbicides were exchanged also between the two countries

E) Methods and Results

Methods:

1. Identify crop resistance to broomrape:

Screening for broomrape resistant or tolerant crop varieties, was based on legume seed bank that exist in Israel (IS) and in the Palestinian Authority (PA) using semi-in vitro systems - Polyethylene bags (PE) which enables quick results within weeks. This approach takes advantage on the availability of legume seed bank that exist in our both countries and the polyethene bag system that was modified and improved by the Israeli team. The combination of these two factors is a novel approach for the screening for resistant or tolerant plants. In PE method, plants are grown on a layer of filter paper in a PE bag. This method allows for visualization of the attachment process so that resistance can be seen at an early stage. Evaluation of resistance of individual plants takes three weeks as compared to three to four months by traditional methods. PA Co-PI was trained in Israel on how to use this technique so that they can do screening in their laboratories, and to ensure that screening procedures are uniform among the research teams. The Israeli and the Palestinian team screened legume species with resistance / tolerance to O. aegyptiaca and O. crenata in their germplasm bank. Lines showed resistant/ tolerance were shared with other project participants.

2. Refine herbicidal methods for broomrape control:

We used low rates of crop selective herbicides (Hershenhorn et al.,1997) for broomrape control.

The research plan of the project was focused on refine herbicidal methods for broomrabe control by using low rates (1.25 + 1.25 and 2.5 gr a. i. /du.) of crop selective herbicides. Greenhouse screening for chickpeas and faba been selective herbicides for *Orobanche* crenata control was conducted in pots under greenhouse conditions. Imazapyr, imazapic (two formulations), imazethapyr, imazaquin, and dimethenamid, were tested at one or two split applications at three weeks interval (Aly et al., 2001).

For refining herbicidal methods, we used in both countries four systems for growing and testing parasites with or without host crops:

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Petri dishes

Petri dishes were used to test parasite seed germination and initial development without the presence of host roots at controlled environment conditions. Parasite seed germination was achieved by induction of GR24. This system served us for screening various herbicides for their effect on germination and development of the parasite seedlings.

Polyethylene bags (PE)

Growing of host plants on GFA paper inoculated with parasite seeds, inserted in Polyethylene bags placed in controlled environment chambers (Goldwasser et al 1997). This method was highly effective for tracking the parasites seed germination in the presence of host roots, development of it's radical towards the host root, penetration and establishment of the parasite in the host tissue, including identification and quantification of various parasite growth stages that were damaged by herbicides. Herbicides can be easily applied into the bags at various growth stages. The method enables continuous microscopic observation without destroying the plant material.

Pots

Pots containing soil artificially infested with parasite seeds were used to screen herbicides, application rates and timing of application all year round in the greenhouse and the net house (Goldwasser et al 1997, Hershenhorn et al 1998).

The most promising herbicides from the screening in Petri dishes and PE bags were tested in pot system.

Field trials

In addition to on-station sites, trials conducted on-farm in naturally infested fields available in Israel. The natural contamination rate of the experimental fields was previously measured, using methods developed by the Israeli team for *Orobanche*. Recently it was succeeded to control *Orobanche* by chemigation of sulfonylurea herbicides directly to the root zone of the host (Hershenhorn et al, 1998), unfortunately this method is not feasible to be used in the PA fields.

3. Agronomic measures (catch crops, trap crops and planting time)

To reduce broomrape seed bank in the soil, we used a trap crop i.e., a plant which causes germination of the parasite but is not parasitized itself, or also a catch crop i.e., a host that is destroyed in an early stage together with the young parasites attached to its roots (Kleifeld et al, 1994).This approach takes advantage of the fact that crop plants

like some pepper varieties induce high rate of parasite seed germination (up to 80%) but is resistant or highly tolerant to parasite penetration. Such crops, called trap crops, can diminish soil seed bank in infested fields. Accordingly, we tested the role of pepper (*Capsicum annuum*) as a trap crop for *Orobanche* control in the greenhouse.

We used also other agronomic measures such as planting time to reduce broomrape seed bank in the soil. Two pot experiments at two different locations were conducted at Newe Ya'ar Research Center. Two sowing dates (November 9, and November 30), three weeks apart, were tested on two chickpea varieties with pots containing soil infested either with O. aegyptiaca or O. crenata.

4. Biological control methods:

Samples of diseased broomrape spikes were collected. Most of the samples were collected from naturally-infested plants growing under irrigated or rain fed conditions.

The fungus was isolated from the diseased broomrape spiders by immersing the diseased tissue taken from the edges of the necrotic areas in 70% ethanol solution for 1.5 min and transferred thereafter to 1% hypochlorite solution for 2.5 min. The pieces were plated on Petri dishes containing Potato Dextrose Agar (PDA). Single spore cultures were obtained by spreading diluted fungal and bacterium spore suspensions on water agar plates. Single colonies that grew on the water agar plates were examined under a stereoscopic microscope. Single hyphae emerging from a single spore was picked up and plated on a PDA plate. The fungus was identified at the Plant Protection and Inspection Services, Ministry of Agriculture and Rural Development, Bet-Dagan, Israel. Isolated fungus was tested in the Polyethylene bag (PEB) system for its ability to cause disease symptoms to *Orobanche ramosa* and *O. aegyptiaca* on tomato, *O. cumana* on sunflower and *O. crenata* on vetch. The microorganism was tested also in greenhouse experiment.

The fungus was grown at 24-25°C in the light and sterilized conditions in Czapek growth media, containing extract of broomrape inflorescences. The hyphae and the spores were blended and 1 mL of filtrate was used for c. f. u. number determination. The filtrate volume was filled to 10^5 c. f. u./mL suspension. The media after filtration was extracted with chloroform at pH=7 and the extract after drying was used for characterization of toxic activity and for purification and identification of toxic metabolites.

Experiments were conducted in pots containing a mixture of sand and vermiculite (1:1 v:v). The soil mixture was mixed with the seeds of O. cumana or O. crenata at concentration of 20

g/L. Soil mixture infected with O. cumana was sown with sunflower seeds, and soil mixture infected with O. crenata - with vetch. Plants of each crop were grown in 10 pots. The soil was then inoculated with a suspension containing hyphae and spores of *F. spp* in a concentration of 10^5 c. f. u. mL-1 (80 mL per pot). The inoculation was conducted twice: two and three weeks after sowing. Tap water was used in a control pots.

Local broomrape populations from both the Palestinian Authority and Israel were screened to identify potential biocontrol agents.

Results:

I. Screening for broomrape resistant or tolerant crop varieties, based on legume seed bank that exist in Israel and in the Palestinian Authority

In this project we first focused on screening of crop resistance (fababean, peas, broadbean, checkpea, soybean, cowpea, lentils, vetch spp. and trifolium spp.) to broomrape.

Several faba bean lines were screened in pots under greenhouse conditions for their response to O. aegyptiaca and O. crenata. No complete resistant response could be observed within the faba bean lines tested (Table 1). However, a few lines demonstrated elevated tolerance toward the parasites. Experiments conducted in Polyethylene bag (PE) (Table 2) on common vetch (Vicia sativa L.): Yovel, Aneta,Milisa,Hifa,Presta,Lolita and on Purple vetch (Vicia atropurpurea Desf.) Popany, Sadot revealed that all vitch lines were capable to germinate parasite seeds on their roots however, two lines Popany and Sadot were resistant to parasitism by two *Orobanche* species: O. aegyptiaca and O. crenata.

In trifolium spp. about 400 lines were screened in pots under greenhouse conditions for their response to O. aegyptiaca and O. crenata. Only three lines (1, 106 and Achziv 2) showed complete resistance to both parasite species (Table 2).

In the Palestinian Authority, local cultivars of tomato, broad bean, soy bean, cow pea, chick peas, common vetch, lentil and bean plants were screened for resistance / tolerance to *Orobanche aegyptiaca* in pots. The results indicated that soybean, cowpea and bean were resistant to broomrape since seeds of the broomrape have not germinated. Tomato, broad bean, chickpea, common vetch and lentils were susceptible to broomrape. Seeds of the parasite germinated, attached to the host roots and established the spikes over the soil. The mean of established spikes were 13.3, 14.5, 14, 8.8, and 6.8 for tomato, broad bean, chickpea, common vetch and lentils respectively (Fig.1A). Furthermore the mean weight of spikes was the highest on tomato (2.1g) and the lowest on broad bean and lentils (1.3g) (Fig.1B). The effect of broomrape on the fresh and dry weight of the hosts was significant. The reduction of

fresh weight was 65.8%, 48.6%, 83.1%, 58.6%, and 55.8%, and the reduction of dry weight was 76.4%, 48.6%, 81.8%, 70.5% and 55.6% for tomato, broad bean, chickpea, common vetch and lentils, respectively compared to the control (Fig.2A&B).

Table 1: Faba bean lines response to *Orobanche aegyptiaca* and *O. crenata* in pots.

Faba been line	*O. aegyptiaca*			*O. crenata*		
	No./plant					
	Shoots	Total[1]	Shoots[2] (%)	Shoots	Total	Shoots[2] (%)
171	1.0	2.6	27	0.6	8.6	7
181	2.2	6.2	26	0.0	20.2	0
186	0.6	7.0	8	0.0	11.6	
192	0.4	11.6	3	0.6	21.2	3
204	0.6	5.6	10	2.0	7.6	21
238	1.4	5.2	21	0.8	17.4	4
559	6.2	15.8	28	2.0	19.6	9
570	0.6	7.0	8	0.2	18.0	1
584	1.2	10.0	11	3.0	20.6	13
592	0	6.4	32	2.0	27.2	7
605	4.4	12.0	22	2.6	12.4	17
608	3.0	17.6	15	0.2	17.4	1
641	4.0	15.0	21	0.6	28.6	21
643	1.4	16.4	8	0.8	24.6	3
664	4.4	16.2	21	0.2	43.6	1
666	1.4	7.4	16	0.4	14.2	3
676	1.4	13.8	9	0.8	17.2	4
680	2.0	11.8	15	0.0	13.2	0
687	3.4	19.2	15	2.4	17.0	12
691	2.6	11.2	19	2.2	10.6	17

[1] The number of parasites found attached to the roots at the end of the experiment.

[2] The percentage of the *Orobanche* shoots from the total parasites found on the roots.

Table 2: Resistance of different varieties and species of Vetch to *O. aegiptica* and *O. crenata* in Polyethylene bag system.

Parasite	Host-plant	Germination %	Parasites No
O. aegyptiaca	Popany	45,6±8[1]	0
	Sadot	69.8±9	8.3±3
	Yovel	55.3±10.1	43.31±2
	Aneta	56.3±6	54.0±4
	Milisa	48±7	21±6
	Hifa	45±7	33±7
	Presta	49±8	17±1
	Lolita	43±9	36±10
	Kelson	33.6±6	40±12
O.crenata	Popany	10±4	0
	Sadot	5±4	3±1
	Yovel	94±6	25.5±13
	Aneta	35±7	59±12
	Milisa	-	-
	Hifa	8±3	17±3
	Presta	3±2	2±1
	Lolita	-	-
	Kelson	-	-

[1] Numbers represents LSD value within each species ($P < 0.05$)

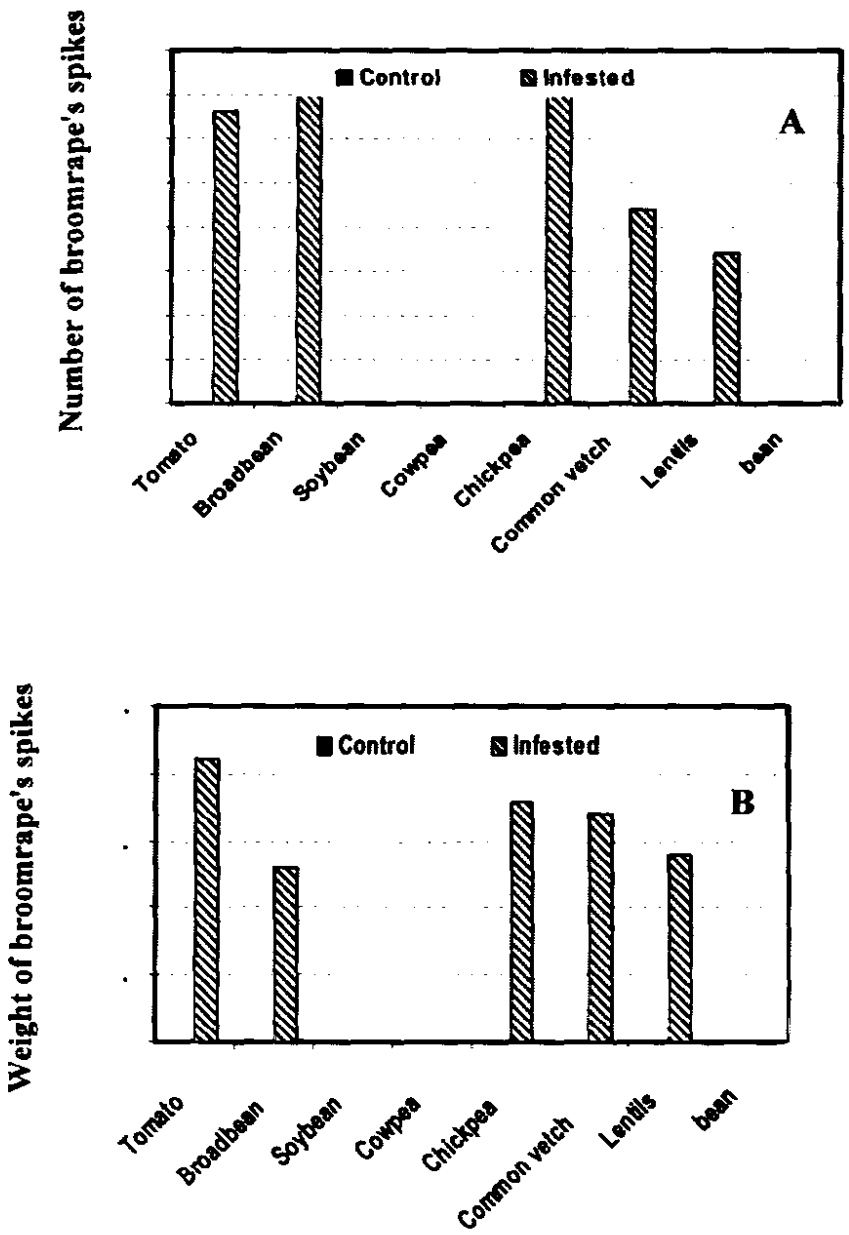

Figure1: Crop resistance for broomrape in pot experiments; (A)-The number of broomrape spikes attached to the crop, and (B)- weight of broomrape spikes.

Local cultivars of (Peas, Sunflower, Lupine, Trifolium) were screened for resistance / tolerance to *Orobanche aegyptiaca* in pots. The results showed that peas were resistant to broomrape but Lupine, sunflower and trifolium were susceptible to broomrape (Figs:3,4)

Overall, excellent progress has been made toward project objectives in the aspects of screening for resistance in both countries.

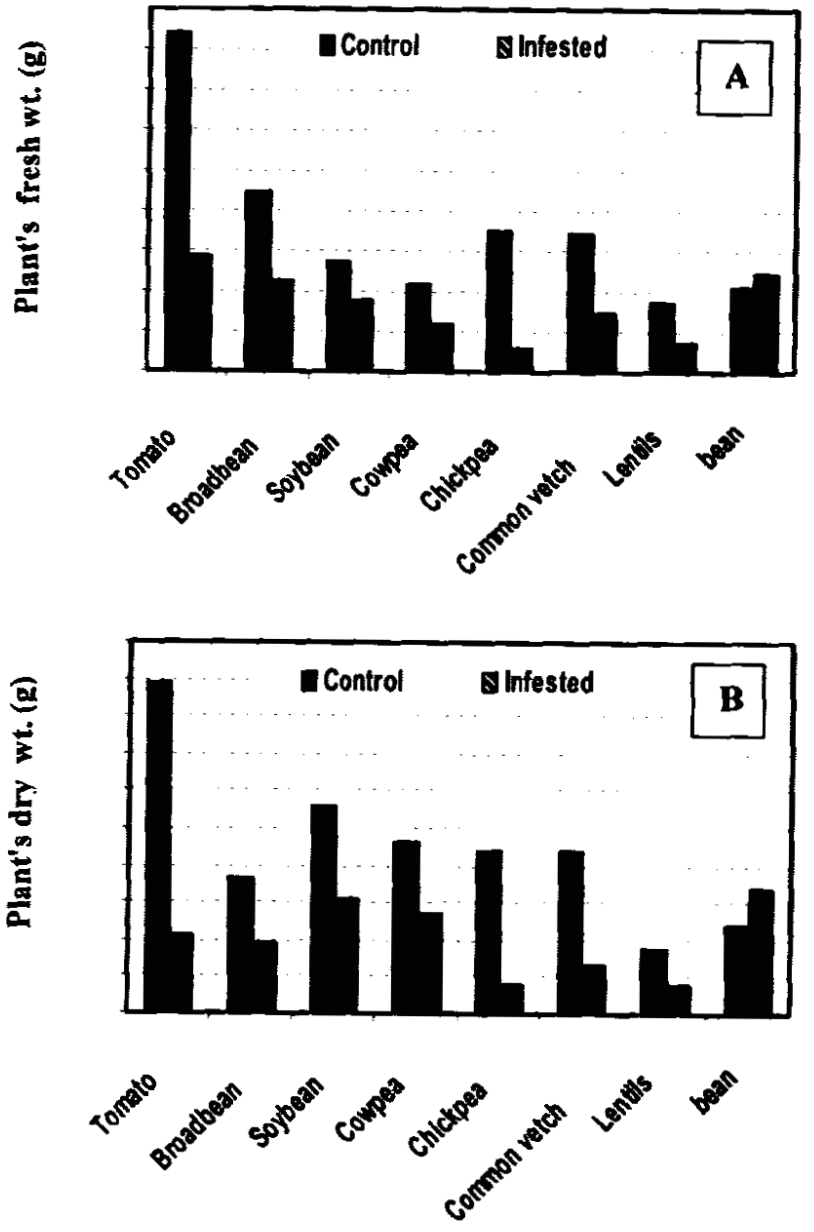

Figure 2: Effect of broomrape's infestation on fresh weight (A) and dry weight of plants (B).

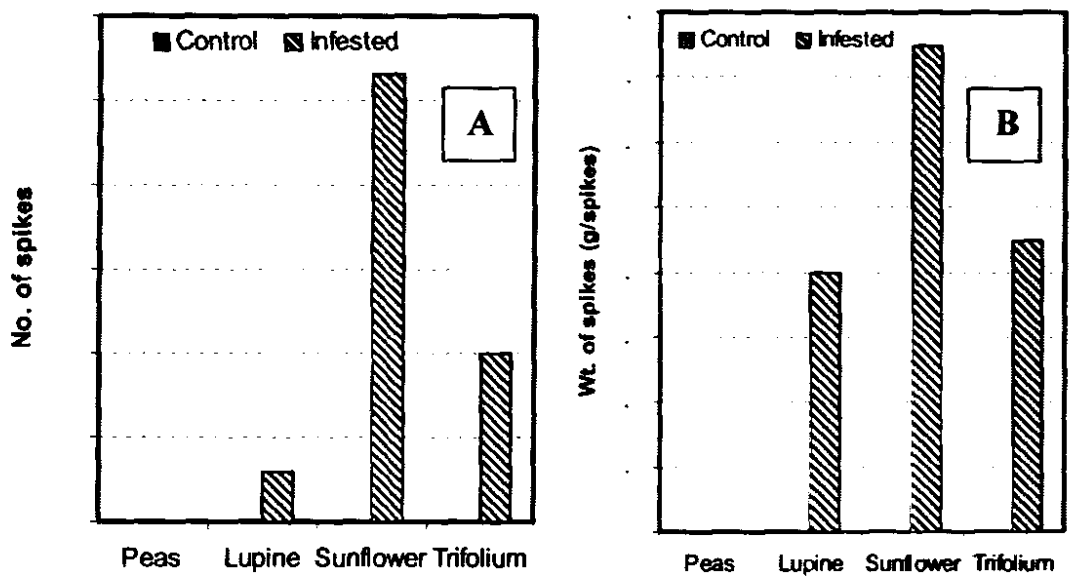

Figure 3: Crop resistance for broomrape in pot experiments; (A)-The number of broomrape spikes attached to the crop and (B) - weight of broomrape spikes.

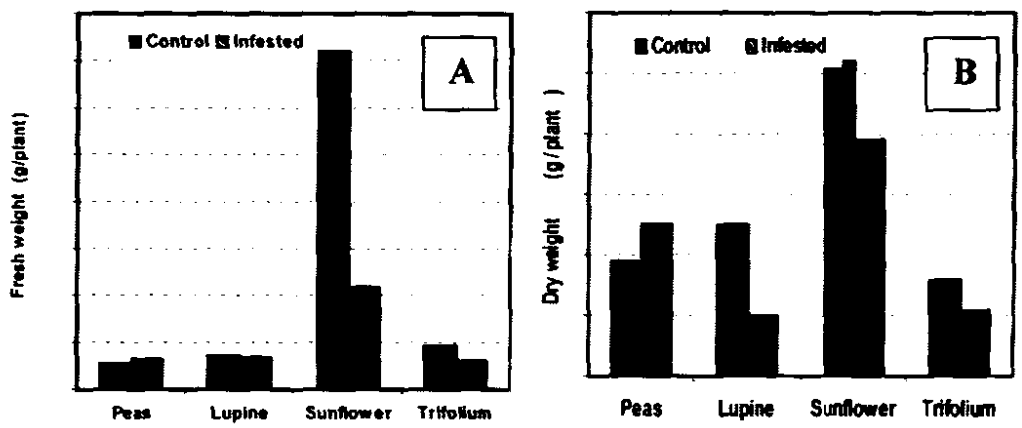

Figure 4: Effect of broomrape's infestation on fresh weight (A) and dry weight of plants (B).

II. Refining herbicidal methods - using low rates of crop selective herbicides for broomrape control.

The research plan was to refine herbicidal methods for broomrabe control by using low rates of crop selective herbicides. Greenhouse screening for chickpeas (Tables 3, 4) and faba been (Table 5), selective herbicides for *Orobanche* crenata control was conducted in pots under greenhouse conditions. Imazapyr, imazapic (Aly et al., 2001) (two formulations), imazethapyr, imazaquin, and dimethenamid, were tested at one or two split applications at three weeks interval. In chickpeas all treatments significantly reduced the number of inflorescences emerged above soil and the number of the total parasites counted on the roots at the end of the experiment (Tables 3, 4). The most effective treatment was imazapyr at 1.25 + 1.25 and 2.5 gr a. i. /du. In faba been all herbicides tested (imazapic, imazethapyr and imazaquin) prevented completely inflorescences emergence above soil. Imazaquin, at the two concentrations tested, significantly reduced the total number of parasites counted on the roots. Overall, excellent progress has been made toward project objectives in selective herbicides for *Orobanche* control.

In faba bean, all herbicides tested (imazapic, imazethapyr and imazaquin) prevented completely inflorescences emergence above soil (Table 5). Imazaquin, at the two concentrations tested, significantly reduced the total number of parasites counted on the roots. The most promising treatments, which need farther attention, were imazapic and imazethapyr (Table 5). More experiments are needed to optimize the use of these herbicides for broomrape control.

Table 3: Chickpea and Egyptian broomrape response to herbicides

Broomrape infested soil

Treatment		Chickpea			*O. aegyptiaca*	
Herbicide	Rate	Vigor[3]	Foliage weight Fresh	Foliage weight Dry	Shoots	Total parasites[1]
	g a. i /du	(0-100)	gr		No./plant	
Imazapyr	1.25	62 c-f[2]	10.9 c-e	2.0 b-e	5.2 b-d	12.2 bc
	1.25+1.25	63 c-f	5.1 ef	1.2 ef	0.0 e	0.2 e
	2.5	50 e-f	7.8 d-f	1.7 c-e	0.0 e	1.0 ef
Imazapic (Oroban)	0.2	80 a-c	13.9 cd	2.8 bc	6.6 bc	9.2 b-d
	0.2+0.2	80 a-c	14.4 cd	2.9 bc	4.8 b-d	11.4 bc
	0.4	80 a-c	12.2 c-e	2.3 b-d	4.8 b-d	10.2 b-d
Imazapic (Cadre)	4.8	60 c-f	8.6 c-f	1.9 c-e	1.6 de	3.2 d-f
	4.8+4.8	26 g	7.1 d-f	1.54 c-e	0.4 e	1.0 ef
	9.2	6h	1.7 f	0.8 e	0.0 e	0.0 e
Imazethapyr	5	74 b-d	16.4 bc	3.3 ab	4.2 b-d	12.0 bc
	5+5	100 a	20.3 ab	4.1 a	4.0 b-d	6.2 b-f
	10	88 ab	22.4 a	4.0 a	4.8 b-d	7.8 b-e
Imazaquin	15	100 a	13.3 cd	2.5 b-d	5.0 b-d	8.8 b-d
	15+15	64 c-f	12.9 c-e	2.4 b-d	0.4 e	3.2 d-f
	30	60 c-f	11.4 c-e	2.1 b-e	3.2 d-f	6.0 b-f
Imazethabenz-methyl	375	52 d-f	8.9 c-e	1.8 c-e	7.4 b	13.2 b
	375+375	100 a	13.5 cd	2.8 bc	2.8 c-e	8.4 b-d
	750	74 b-d	11.1 c-e	2.3 b-d	5.0 b-d	9.2 b-d
Dimethenamid	135	80 a-c	12.4 c-e	2.6 b-d	4.2 b-d	9.6 b-d
	135+135	70 b-e	11.4 c-e	2.4 b-d	1.4 de	3.6 d-f
	270	46 f	6.4 d-f	1.5 c-e	2.0 de	5.0 c-f
Control		58 c-f	6.9 d-f	1.5 c-e	11.0 a	20.2 a

[1] The number of the total parasites found attached to the roots after washing the soil from the roots at the end of the experiment.

[2] Means within a column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's new multiple range test.

[3] Vigor evaluation scale from 0 = dead plants to 100 = vigorous plants.

Table 4: Chickpea and Egyptian broomrape response to herbicides

Non infested broomrape soil

Treatment		Chickpea		
Herbicide	**Rate gr a. i./du**	**Vigor[1] 0-100**	**Foliage weight Fresh**	**Dry**
			gr	
Imazapyr	**1.25**	96 a-c[2]	14.8 a-g	2.8 b-e
	1.25+1.25	28 e	6.5 hi	1.6 e-h
	2.5	50 d	8.0 g-i	1.6 e-h
Imazapic (Oroban)	**0.2**	80 c	18.5 a-f	3.4 a-c
	0.2+0.2	84 bc	20.2 a-d	3.8 ab
	0.4	84 bc	16.4 a-f	3.1 a-d
Imazapic (Cadre)	**4.8**	50 d	4.7 i	1.0 gh
	4.8+4.8	32 e	6.7 hi	1.3 f-h
	9.2	22 e	2.3 f	0.9 g
Imazethapyr	**5**	100 a	22.2 ab	4.2 a
	5+5	100 a	22.6 a	4.1 a
	10	100 a	18.7 a-f	3.3 a-c
Imazaquin	**15**	98 a	13.8 c-h	2.3 c-f
	15+15	92 a-c	13.2 d-h	2.3 c-f
	30	80 c	11.0 hi	1.9 d-h
Imazethabenz-methyl	**375**	96 ab	21.3 a-c	3.7 ab
	375+375	100 a	16.5 a-f	3.2 a-d
	750	100 a	16.6 a-f	3.1 a-d
Dimethenamid	**135**	100 a	14.6 b-g	2.7 b-e
	135+135	80 c	13.6 c-h	2.5 b-f
	270	80 c	11.5 e-i	2.2 c-g
Control		90 a-c	19.2 a-e	3.8 ab

[1] Vigor evaluation scale from 0 = dead plants to 100 = vigorous plants. Observation made at harvest.

[2] Means within a column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's new multiple range test.

Table 5: Faba been and *Orobanche crenata* response to herbicides

Treatment	Rate	Faba been		*Orobanche crenata*	
		Foliage Fresh Weight		Shoots	Total parasites[1]
	gr. a. i./du	gr		No./plant	
Broomrape[2]		-	+		
Imazapic	**1.25 + 1.25**	219a	183a	0.0b	34.8a
Imazapic	**1.25 + 2.5**	171a	196a	0.0b	19.8ab
Imazethapyr	**1 + 1**	233a	205a	0.0b	21.8ab
Imazethapyr	**2 + 1**	233a	222a	0.0b	17.6ab
Imazaquin	**1.5 + 1.5**	227a	149a	0.0b	14.2b
Imazaquin	**3.0 + 1.5**	226a	168a	0.0b	12.0b
Control[3]		191a	150a	3.6a	36.6a

[1] The number of the parasites recorded on the roots of the infected plant at the end of the experiment.

[2] Plants grown in non-infested broomrape soil (-); Plants grown in infested broomrape soil (+).

[3] Untreated plants grown in non-infested broomrape soil.

In Pea: Greenhouse screening for Pea and lentil selective herbicides for *Orobanche crenata* control was conducted in pots (10 replicates for each treatment) under greenhouse conditions. Imazapic, imazethapyr, imazaquin, were tested at two split applications at three weeks interval. In Pea (Table 6) the herbicides applied to the pea plants prevented effectively broomrape inflorescences emergence above soil. No reduction of the total number of the parasites counted on the roots was recorded. However, the fresh and the dry weights of the treated plants were not affected by the herbicides or the young parasites that were attached to the roots (Table 6).

In Lentil: Imazethapyr, imazaquin and imazapic at 1.25 + 2.5 gr a. i./du were highly effective and reduced the number of inflorescences that emerged above the soil without affecting the number of the parasites on the roots (Table 7). Imazethapyr and imazaquin didn't reduce the fresh and dry weight of the treaded lentil plants and protected them

from broomrape damage. Imazapic treatment didn't prevent the damage caused to the lentil plants by the parasites (Table 7).

Table 6: Pea and *Orobanche crenata* response to herbicides

Treatment Rate	Pea		*Orobanche crenata*	
	Foliage Fresh Weight		Shoots	Total parasites[1]
gr. a. i./du	gr		No./ plant	
Broomrape[2]	-	+		
Imazapic 1.25 + 1.25	173a	110a	7.4b	54.4a[3]
Imazapic 1.25 + 2.5	228a	104a	2.4c	57.2a
Imazethapyr 1 + 1	192a	138a	0.6c	57.8a
Imazethapyr 2 + 1	169a	149b	0.2c	59.6a
Imazaquin 1.5 + 1.5	185a	161a	0.6c	32.2a
Imazaquin 3.0 + 1.5	184a	117a	0.0c	64.3a
Control[4]	165a	75a	14.8a	54.6a

[1] The number of the parasites recorded on the roots of the infected plant at the end of the experiment.

[2] Plants grown in non-infested broomrape soil (-); Plants grown in infested broomrape soil (+).

[3] Means within a column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's new multiple range test.

[4] Untreated plants grown in non-infested broomrape soil.

Table 7: Lentil and *Orobanche crenata* response to herbicides

Treatment Rate	Lentil		*Orobanche crenata*	
	Foliage Fresh Weight		Shoots	Total parasites[1]
gr. a. i./du	gr		No./ plant	
Broomrape[2]	-	+		
Imazapic 1.25 + 1.25	161ab	95a	5.4b	62.2a[3]
Imazapic 1.25 + 2.5	154ab	95a	9.7b	56.2a
Imazethapyr 1 + 1	171ab	157b	0.2b	64.0a
Imazethapyr 2 + 1	160ab	159b	0.4b	68.0a
Imazaquin 1.5 + 1.5	174a	147b	0.0b	58.0a
Imazaquin 3.0 + 1.5	124b	126ab	0.3b	53.8a
Control[4]	182a	89a	15.2a	74.6a

[1] The number of the parasites recorded on the roots of the infected plant at the end of the experiment.

[2] Plants grown in non-infested broomrape soil (-); Plants grown in infested broomrape soil (+).

[3] Means within a column followed by the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's new multiple range test.

[4] Untreated plants grown in non-infested broomrape soil.

During this period of the project, our partner in the Palestinian Authority had also initiated experiments refining herbicidal methods. Actually, these methods had been transferred to our partner since they were proved to be very effective in the Israeli fields. Three herbicides; Glean® 75% WG (Chlorsulfuron), DU PONT DE NEMOURS CO.; Amber® 75% WG (Triasulfuron) CTS Co.; and Scepter® 180g/L SL (Imazaquin), AGAN were used for evaluating their efficiency in controlling broomrape in tomato fields first. Preliminary results showed that the herbicides, Glean, Amber and Scepter were very effective against broomrape in tomato fields at the concentrations (5-50 *ppm* a.i). The percentage of dead broomrape was 90%-100%. However, herbicides applications affected plant's growth at high concentrations (≥ 10 *ppm* a.i). The experiments was repeated again using the three herbicides at different concentrations (0,

1, 3 , and 5 ppm a.i) in open commercial irrigated field. The experiment design was completely randomized block. Plants in each plot were sprayed by 200 ml/plant of each herbicide. The number of dead spikes and the tomato growth was evaluated after two weeks. Results showed repeatedly that herbicidal applications affected significantly broomrape at concentrations (≤5 *ppm*). The best results were recorded by Glean, Amber, and Scepter, respectively. Numbers of dead spikes and weight percentage increased as the concentrations of herbicides increase (Fig.5).

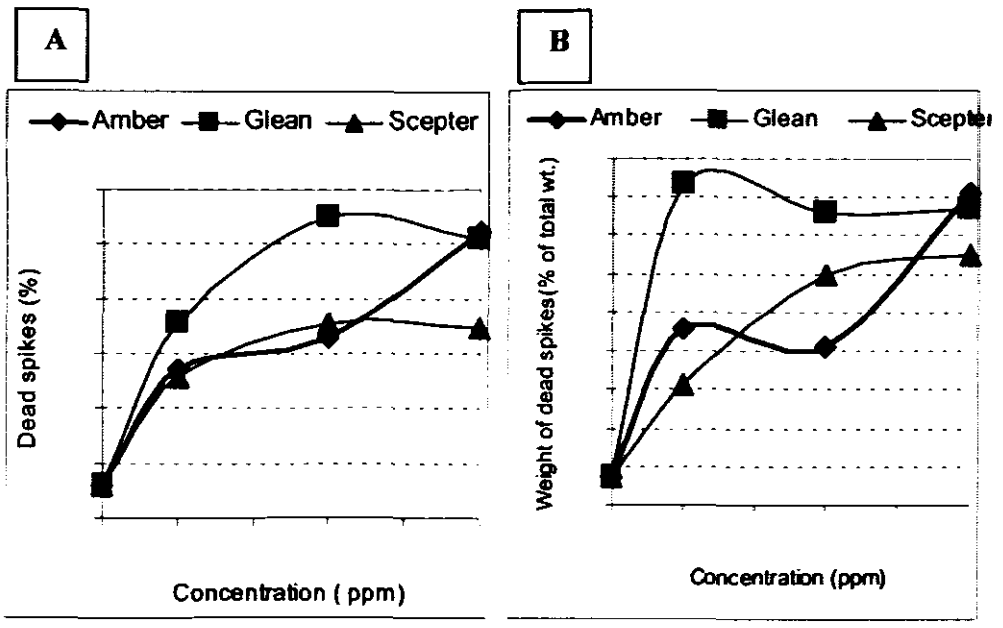

Figure 5: Effect of herbicides on broomrape dead spikes number (%) (A) and weight of dead spikes as percentage of total wt. (B) on tomato plants growing in irrigated open field during the summer of 2005.

III. Development of agronomic measures (catch crops, trap crops and planting time)

Effect of Chickpea sowing dates on parasitic weeds control

Two pot experiments at two different locations were conducted at Newe Ya'ar Research Center. Two sowing dates, three weeks apart, were tested on two chickpea varieties with pots containing soil infested either with *O. aegyptiaca* or *O. crenata*. The results of the two experiments were similar; therefore only the results of one of them will be presented. Foliage fresh weight of 'Hadas' variety grown in non-infested broomrape soil

at the two sowing dates was not significantly deferent. A significant decrease in chickpea foliage fresh weight was observed in the plants grown in *O. aegyptiaca* and *O. crenata* infested soil on both sowing dates as compared with the non-infested control. However, the damage caused by *O. aegyptiaca* at the latter sowing date (November 30) as expressed in foliage fresh weight was more severe than the damage caused by *O. crenata* .Almost a complete loss of the 'Yarden' variety plants grown in *O. aegyptiaca* infested soil at the two dates tested (November 9 and November 30). At the latter sowing date, 64% reduction in foliage fresh weight of the chickpea plants grown in *O. crenata* infested soil was recorded. Growth parameters such as plant vigor, plant height, and number of broomrape shoots emerged above soil level were recorded periodically. No significant deference between the treatments and the non-infested control could be observed and therefore the data is not shown.

Our results indicate that altering chickpea sowing dates has no practical advantage in preventing O. *aegyptiaca* and *O. crenata* damage to the chickpea plants. On the other hand, relative higher tolerance of var' 'Hadas' toward both broomrape species as compared to the var' 'Yarden' could be observed. Based on these results we can hypothesis that screening for more resistant chickpea varieties is more likely to succeed than altering sowing dates. More experiments with a wider variety collection are needed in order to establish this hypothesis.

We had also tested the role of pepper (*Capsicum annuum*) as a trap crop for *Orobanche* control in the greenhouse. Our preliminary results showed that some pepper varieties (sweet and hot) (data not shown) induced germination of *Orobanche* seeds but *Orobanche* failed to infest the host plants. Although, pepper as a summer crop could not be effective for winter crops such as legumes, but it could be used and adapted as a trap crop in an integrated control system in the summer season for summer crops.

IV. Biological control methods – bioherbicides

Although biological control methods for broomrape are limited but they could contribute in reduction of broomrape seeds through an integrated approach.

In our project we focused on biological control approaches mainly isolation of a new pathogen of parasitic plant *Orobanche* sp. A strain of Fusarium moniliforme Sheldon was isolated from *Orobanche* cumana, parasitizing sunflower plants. The pathogenicity of the fungus was tested in polyethylene bag system (PEB) (Fig. 6) and in pots in the

greenhouse (Fig. 7). Fusarium moniliforme caused disease symptoms on *Orobanche aegyptiaca* , *O. ramosa* on tomato and *O. cumana* on sunflower.

F. moniliforme, grown on Czapek growth media with addition of broomrape extract, produced phytotoxic metabolite that caused large necrotic areas on broomrape inflorescences and leaves of many crops in the leaf puncture bioassay. The extract of the fungus growth media also caused complete mortality of O. aegyptiaca and O. cumana germinating seeds. The main toxic metabolite was isolated and identified by spectroscopic methods as Fusaric acid. The compound identity was also confirmed by conversion into the corresponding methyl ester and comparison against authentic Fusaric acid on TLC.

In parallel, our colleagues from Hebron University were able to collect more than 100 fungi isolates. Most samples were collected from naturally-infested tomato plants growing under rain fed conditions. Suspected pathogens were isolated on semi-selective medium, purified and sub cultured on PDA medium. The identification of pathogens showed that most of them are *Fusarium* spp. Amsellem et al. (2001b; 2001b) and *Rhizoctonia solani*, in addition to other fungi which are under investigation. There were 109 isolates of *Fusarium* and 14 isolates of *R. solani*. The pathogenicity of the strains was compared with the identified isolates *Fusarium oxysporum* strain EId (CNCM-I-1622) and *Fusarium arthrosporioides* strain E4a (CNCM-I-1621), obtained from the Collection of Nationale de Cultures de Microorganismes (CNCM), Institute Pasteur, Paris. Both strains are reported to be pathogenic to *O. aegyptiaca* (Amsellem et al., 2001), and none pathogenic to any of a variety of commonly cultivated vegetables. The identified "fungal pathogens" were investigated on broomrape and tomato growing in pots under greenhouse conditions. The dead spikes were evaluated after two months. The results (Fig. 8) indicated that there was significant variation in the pathogenicity of the isolates to broomrape; the most effective isolates in the first set of experiments were Farth, Fu30, Foxy, Fu5, Fu45, and Fu119, and in the second one were Fu30, Fu87, Fu 20, Farth, Foxy, and Fu119, respectively.

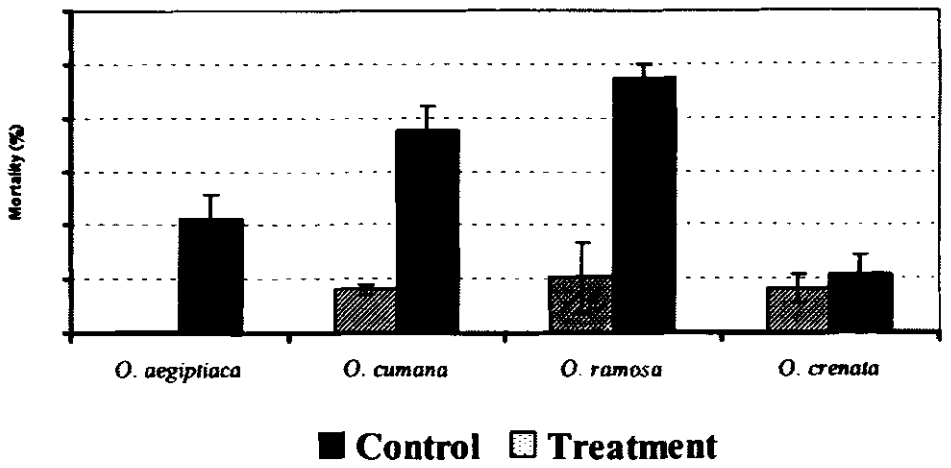

Figure 6: Effect of *F. moniliforme* inoculation on total mortality of *Orobanche* spp. in PEB.*

* In the PEB, 15 ml of *F. moniliforme* suspension at concentration of 10^5 c.f.u. per 1 ml. were sprayed on *O. aegyptiaca, O. ramose*, *O. cumana* and *O. crenata* . Experiments were conducted twice with nine replicates. The statistical analysis was conducted using ANOVA by means of the JMP Software, version 5.0 (SAS Institute Inc. USA). Standard deviations of means is indicated by the vertical lines

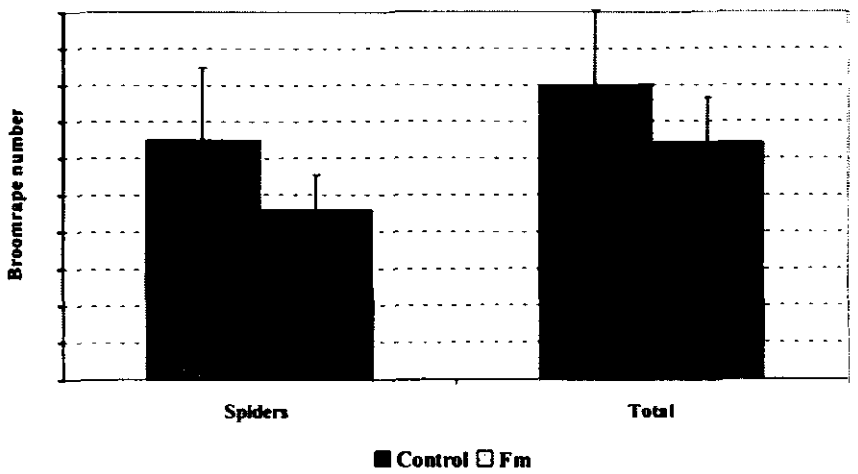

Figure 7: Effect of soil inoculation with *F. moniliforme* on *O. cumana* mortality in greenhouse experiment*

* Pots containing *O. cumana* infested soil was inoculated with fungus suspension at concentration of 10^5 c.f.u. per ml, 80 ml per pot. The experiment was conducted twice, each time with five replications. The statistical analysis was conducted using ANOVA by means of the JMP Software, version 5.0 (SAS Institute Inc., USA). Standard deviations of means is indicated by the vertical lines

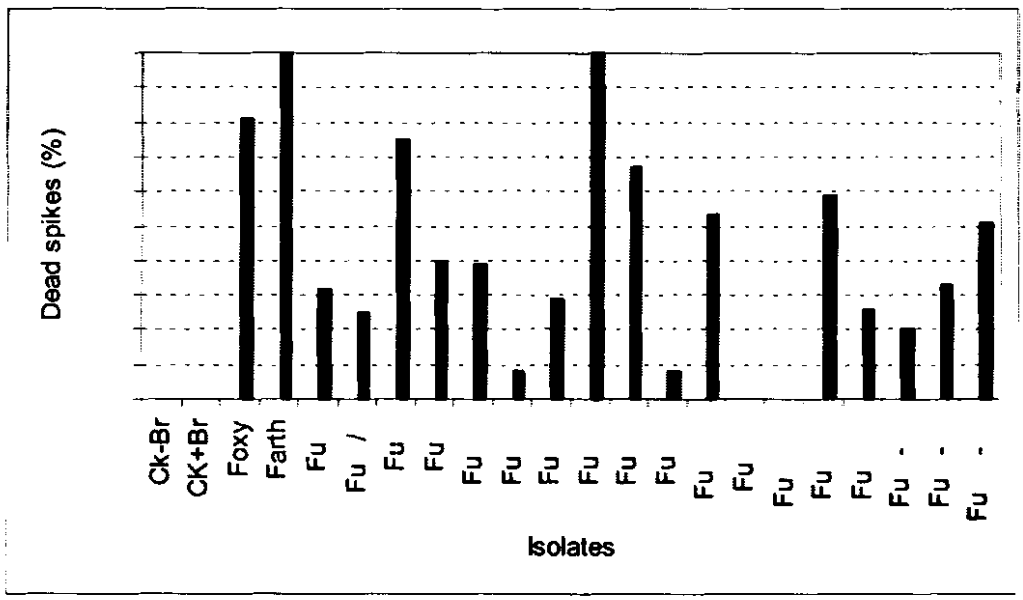

Figure 8: Effect of *Fusarium* spp. isolates on broomrape of tomato plants growing in pots and incubated under greenhouse conditions.

F) Impact, Relevance, and Technology Transfer:

Despite the political situation prevailed in the area, the interaction between Palestinian and Israeli researchers was a joint effort and an integral part of this project. During the project, Dr. Barakat (co-principal investigator) from the Palestinian side visited Israel every year for training and to review progress, collaborate on experiments and discuss plans. Dr. Barakat was responsible for the laboratory and field experiment work at the Palestinian Authority. Following consultation with the Israeli team in practical subjects of cropping legumes in the Palestinian Authority he was responsible for collaboration with the local Agricultural Administration for application of outstanding results from the project in demonstration plots at farmers' fields. Both countries will benefit from the information, technologies and management practices developed by the project. The information will be disseminated by the Palestinian and Israeli Ministries of

Agriculture. People directly involved in the project, researchers and students, are also regularly in direct contact with growers and the grower's organizations, and will be involved in dissemination of information by lectures, visits and articles in trade journals. Impact of the project's results will have a direct effect on production, by reducing broomrape infestation in legume fields, thereby lowering production costs and mitigating environmental impact, without compromising with crops quality. Primary beneficiaries of the information generated by this project will be the farmers of the Middle East. While the increased food and export-crop production expected to consumers and society as a whole, the focus of technology development will also realize result from this project and distribution will be the farmers and their local communities. These collaborations yielded development of good personal contacts and strong friendships. The current project will extend the fields of collaboration and bring additional people into contact.

G) Project Activities/Outputs:

According to our proposal our plan was to transfer techniques developed in Israel to our partner in the Palestinian Authority by training Palestinian scientists and technicians in Israeli facilities and following their activity in adapting it to Palestinian farming and environment. Originally, we planned to train a Palestinian Ph.D. student who will carry out most of the research after a training period in Israel. Israeli scientists should visit the Palestinian Authority periodically for planning, discussing and supervising research with the Palestinian collaborators, especially in the field experiment seasons. During implementation of the project, much collaborative work should be done including workshops, training sessions and annual meetings. Due to political obstacles prevailed in the area, security and difficulties to cross the borders between the two countries, some of these activities (training a Palestinian Ph.D. student in Israel, workshops, training sessions and annual meetings) were not accomplished. Despite this situation, cooperation, training and information transfer had been established through the visits of our co-principal investigator Dr. Barakat to Israel during the time of the project. Techniques that were recently developed in Israel were transferred to the target country (Palestinian Authority) by the assistance of Dr. Barakat and his students through his annual meetings with us every year on summer season at Newe Yaar Research Center. Despite governmental and infrastructural limits our current project is unique because we planned to extend our research results to the farmers. We anticipate that outstanding

results originated from this project was already transferred by Dr. Barakat to our partners in the Palestinian Authority and distributed through conventional agricultural extension networks to the extent possible.
Recently, project participants are working on preparation of two joint scientific publications that include co-authors from Israel and the Palestinian Authority.

H) Project Productivity:

Overall, excellent progress has been made toward project objectives in the aspects of: screening for resistance in both countries, refining herbicidal methods and biological control methods. On the other hand some management and control measures for parasitic weeds such as crop rotation, trap and catch crops, application of soil fumigants or solarization to decrease parasite seed bank in infested fields, do not fit small scale farms in the target country because of socioeconomic and cultural reasons.
Never the less, the results and experience of *Orobanche* control using resistant lines, low rates of potential herbicides and the bioherbicide *Fusarium* spp. acquired recently by both the Israeli and the Palestinian teams will serve as a basis for adaptation and further development of integrated broomrape control. Additionally, workshops, training sessions and annual meetings were not accomplished due to escalation in security situation and political obstacles prevailed in the area.

I) Future Work:

Integrated *Orobanche* management suggests the use of two or more control methods for managing the parasite in a particular field (Joel et al., 2006). Taking into account the present status of each of the control measures described above, the most practical and feasible combination would be the use of resistant cultivars together with chemical, biological and cultural control.

As a future plan, the following remarks should be taken in account:

1. Development of integrated measures using outstanding results and developed technology originated from the project aimed to control *Orobanche* in legume fields.
2. Adoption of technology is often influenced by the socioeconomic and institutional constraints of the targeted population (Schultz, 1964). Therefore a

plan for social science research and farmers education is required for further success in combating these vicious parasitic weeds.

3. Both the Israeli and the Palestinian teams will continue working on the biological control aspect – identification, isolation and testing the efficacy of those isolates on *Orobanche* control in legumes and exchange the most efficient isolates between the two countries.

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