PD-ABK-222

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October 17, 1994

Mr. Edward Lijewski
MERC Project Officer Global Bureau
(G/HCD/PP) Room 606, SA-18
U.S. Agency for International Development
320 Twenty-First Street, N.W.
Washington, DC 20523 - 0223

Dear Mr. Lijewski:

Reference: HNE-0158-G-00-3060-00, "Multipronged Approaches to Eliminating Crop Devastation by Parasitic Weeds"

Pursuant to a contract agreement between US AID (Granting Agency) and Virginia Polytechnic Institute and State University (Grantee), dated September 13, 1993, I am enclosing five copies of our first annual Technical/Progress Report for the above referenced Tri-National research project on *Orobanche* with Israel and Egypt. The reporting period covered is September 13, 1993 through September 12, 1994.

Financial Reporting and Audits on the Grant will be handled by the Office of International Development and the Office of Sponsored Programs at VPI & SU, the "lead institution", in collaboration with the Sub-Contracts in Israel and Egypt.

Should further clarification be necessary on any aspect of this report, please let me know. Many thanks for your cooperation and support in this research effort.

Sincerely yours,

Chester L. Foy

Chester L. Foy, Professor
Scientific Coordinator (U.S.)
for all Project Coordinators

Jonathan Gressel, Professor
Scientific Coordinator (Israel)

Esmat A. Hassan, Professor
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Proposal for a Joint Egypt, Israel, USA
Tripartite Regional Cooperation Project
to A.I.D.

Multipronged Approaches to Eliminating Crop Devastation by Parasitic Weeds

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First Annual Report

Joint Egypt, Israel, USA A.I.D. Tripartite Regional Cooperation Project

Submitted to A.I.D.

Multipronged Approaches to Eliminating Crop Devastation by Parasitic Weeds

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September, 1994

INTRODUCTION

The devastating effects of root parasitic weeds and the limitations of current attempts to alleviate these problems are both well recognized. The present project was conceived and funded to investigate multiple approaches (including some innovations) toward eliminating, or at least minimizing, the crop losses now caused by broomrape (*Orobanche* spp.). Also, hopefully, by future extension of the principles learned, the losses caused by other important parasitic weeds, e.g. witchweed (*Striga* spp.) and dodder (*Cuscuta* spp.), may be somehow mitigated as well.

The overall **Research Objectives** of this project are those listed in the research proposal (p. I-3), as follows:

1. To conduct comprehensive surveys of host ranges of species of *Orobanche* in Egypt and Israel;

2. To develop a service for prediction of broomrape potential in the field from information on levels of infestation and cropping practices;

3. To identify different broomrape populations within a species (races) that may have different host ranges or different degrees of virulence, by implementing a molecular approach for detecting species, races, and possibly crosses among them;

4. To analyze several enzymes, amino acids, and/or proteins in the major species and/or strains of broomrape in comparison with those in host or trap crops;

5. To study seasonal effects on broomrape infestation to escape infection by altering planting dates;

6. To develop methods to use plant growth regulators as therapeutic agents to protect crops from damage and to modify crop phenology to "escape" broomrape infestation;

7. To screen new herbicides for selective broomrape control in selected crops both at the recommended rates and at lower rates;

8. To provide methods of *Orobanche* control using present and future herbicides together with "safeners" and/or plant growth regulators;

9. To determine by bioassay, radiotracer techniques (autoradiography, liquid scintillation spectrometry, etc.), chemical and/or chromatographic analysis, the patterns and mechanisms of herbicide absorption, translocation, or

distributional fate and phytotoxic action in selected host crops and broomrape species. To determine the effects of various adjuvants on herbicide performance against broomrape;

10. To elucidate the basis of nitrogen suppression of *Orobanche* and unsuccessful penetration of haustoria into trap plants.

11. Added objective (referred to later): Major attempts have been, and will be, made to obtain and/or appropriate genetically engineered resistances, to either the parasitic weed directly or to herbicides that appear to be promising for its control. Success in the latter area will be dependent on the availability and willingness of industry to provide either transgenic plants or gene constructs that are suitable for incorporation into important crop species grown in the region.

The **Working Plans** and **Innovations** designed in an attempt to accomplish these objectives are also given in the proposal (pp. I-3 through 8). Unless specified otherwise in this annual report, the 6-months and 18-months work plans for each of the various activities and sub-activities may be assumed to remain unchanged.

Although the research project was officially approved by letter dated September 13, 1993, there were several seemingly unavoidable delays in actually getting the funds into the hands of project personnel. Thus, research in many areas could not begin in earnest until about February or March, 1994, when most of the first 6-months reporting period was past. The retroactive starting date (unknown in advance) and the even-later issue of sub-contracts severely affected employing personnel and procuring needed equipment and supplies. Also, some required laboratory space and greenhouse renovations were not finished during the first 6 months and thus major capital purchases were delayed, partly due to problems in coordinating transfer of funds for major purchases.

Despite the somewhat late start, however, several important project-related matters were pursued, and significant and encouraging progress may now be reported in several areas. The collaborative effort and integrative nature of the various sub-projects of this multipronged, tri-national research project should be evident from both the **Research Objectives** and the contents of this **First Annual Report**.

The various research activities are itemized in the order shown in the research proposal, and in accordance with the list in the contract document, Attachment 1, Schedule Section E. Reporting Requirements E.2.(6)(2) Technical/Progress Reports --- annual reports, which accompanied the grant approval letter from Timothy T. Beans, Grant Officer of AID, addressed to H. T. Hurd, Director of Sponsored Programs at VPI & SU.

TECHNICAL/PROGRESS REPORTS

First annual report covering the period September 13, 1993 to September 13, 1994.

Activity I. **Detection-Monitoring-Correlating Damage, Cultural and Chemical Control Mechanisms**

Egypt - E. A. Hassan (Principal Investigator)

A. Infestation Surveys/Crop Losses by *Orobanche* and Novel Herbicide/Protectant/Plant Growth Regulators Protection of Crops from Damage.

1. Survey of geographical distribution and host range of the different broomrape species. Work plan section 3.1-3.1.1.

   a. The N.R.C. group surveyed 5 governorates (Delta - Middle Egypt - Upper Egypt), all old soils except one sandy new reclaimed location (Nobaria), in the winter season 1993/94.

   b. Survey started October 1993 before *Orobanche* emergence to interact with farmers, land owners, and extension personnel to collect agricultural and social information, and to choose a fixed field for the next phase of the work plan (season 1994/1995).

   c. Photographs (color slides) were taken and taxonomical samples of *Orobanche*/host spp. were preserved. All were kept for demonstration and for the expected performance of section 3.1.2, 3.2.2, and 3.3 of the work plan in cooperation with the Israeli group (see Appendix).

   d. Governorates: (see Table 1).

Delta:	North	: El-Beheira	
	Middle	: El-Gharbia	7 Districts
	East	: El-Dakahlia	12 Districts
Middle Egypt		: Banysweif	7 Districts
Upper Egypt		: Assuit	6 Districts

Table 1. *Orobanche* parasitism comparative data from Delta and Upper Egypt.

Records	North Delta El-Behaira	Middle Delta El-Gharbia	Upper Egypt-Assuit
Hosts	Peas *See information sheet page.	Eggplant, Egyptian clover, cabbage, cauliflower, tomato, potato **Vegetable Hosts.	Faba bean, Egyptian clover, carrot, tomato, cucumber, cumin, chick peas, lupin, sweet peas, chamomile, garden nasturium, corn panic grass
Soil type	Sandy new reclaimed	Heavy clay-old soil	Heavy clay old-new reclaimed (faba bean) (chamomile)
Sowing dates	Oct. 15	July-Aug.:Cruciferous Oct.-Nov.:Tomato, eggplant Sept.-Oct.:Potato	Sept., Oct., Nov., and Feb. for chamomile and sweet peas
Orobanche appearance	December	Oct., Nov., Jan., Mar.	Jan., Feb., Mar.
Near crops	Onion, wheat	Corn, zucchini, lettuce	Onion, wheat, cucumber, *See Hosts, above
Previous crops	Ground nut, water-melon, cowpeas, onion, E. clover, faba bean	Clover, corn	Corn, cotton, sesame
Infection %	86	30 to 80	18 average
Irrigation	Mist irrigation	Surface irrigation	Surface irrigation

Agricultural information: Peas attacked by *Orobanche* in El-Hussain Village at Nubaria (private land) during 1993/1994

1-	Host	:	Peas var. 'Lincoln'
2-	Area	:	Three feddans
3-	Soil type	:	Sandy soil, new reclaimed
4-	Time of transplanting	:	Oct. 15, 1993
5-	Plowing	:	Two times before planting
6-	Type of planting	:	Ridge planting
7-	Rate of planting	:	15 kg seeds per feddan
8-	Source of seeds	:	Stock from the grower
9-	Rhizobium	:	Seeds artificially inoculated
10-	Distance of planting	:	20 cm
11-	Farmyard manure	:	Not added in that season
12-	Calcium superphosphate	:	200 kg per feddan
13-	Nitrogen (sulfate)	:	200 kg per feddan
14-	Nitrogen (nitrate)	:	100 kg per feddan
15-	Potassium sulfate	:	350 kg per feddan
16-	Irrigation	:	Mist irrigation every four days
17-	Diseases	:	Powdery mildew, rust
18-	Disease control	:	Sprayed with Bayleton
19-	*Orobanche* registered	:	Appeared in Dec. 1993
20-	Pod collection	:	Started Jan. 1994 (by hand)
21-	Yield	:	1.5 tons per feddan
22-	Yield before *Orobanche* appearance	:	5 tons per feddan
23-	Previous crops	:	Ground nut, watermelon var. 'Gorma', cowpea, onion, Egyptian clover, peas, and broadbean
24-	Near crops	:	Onion and wheat

2. Use of plant growth regulators with herbicide(s) to control broomrape. Work plan 3.7.

Safener Tests Against Glyphosate and Butralin (Amex) Herbicides. The group on safener synthesis provided a water soluble compound (SG-91) for tests.

Personnel:	E. S. Hassan	N. Y. Aboul Enein
	M. A. Abd Rabbo	Aida El-Azzoni
	K. Gad	Synthesis
	G. M. Naim	

Our previous laboratory bioassay test results showed that glyphosate mainly arrested root growth in different plant species, i.e. wheat, maize, sorghum, barley, lentils, mung bean, peas, and cucumber. *Orobanche* infection as well caused extreme physiological root stress to the host. Therefore, our objective in testing compounds as safeners against glyphosate, the main herbicide proven to control *Orobanche* in the field, and also against butralin, a dinitroaniline herbicide used to control *Cuscuta* on different plant species, was to detect the efficiency of a given compound on increasing root growth.

a. Germination Bioassay Laboratory Test. (Figure 1).

Wheat was chosen for the test because of its proven susceptibility to glyphosate. However, any potential effect of the safener compound can be easily detectable, particularly on the roots.

The water soluble compound (SG 91) was tested against glyphosate at 500 ppm on wheat cv. Sakha 69 (local), and from 5 to 1000 ppm on cucumber cv. Beit Alfa Hybrid (imported) (Figure 1). The seeds were soaked sequentially in glyphosate, followed by the safener for 30 min each.

Results were recorded on 2-wk-old seedlings. SG 91 at 500 ppm resulted in maximum counteraction to glyphosate inhibition of root growth in wheat, whereas for the shoots a concentration of 100 ppm was sufficient. In cucumber (dicot), 20 ppm of the safener compound for the roots and 5 ppm for the shoots were sufficient.

b. Evaluation of Vita 2000 and a commercial phytokinin compound against glyphosate on pea plants in the greenhouse (Table 2).

The seed dressing compound Vita 2000 and a commercial phytokinin from Tilco-Biochemie/Germany were applied. Vita 2000 was applied as 1/1 (w/v) as seed dressing before cultivation. Phytokinin was applied as a soaking treatment (30 min) in 2/1000 of the stock solution before cultivation; or as foliar spray after cultivation and 48 h before the glyphosate spray.

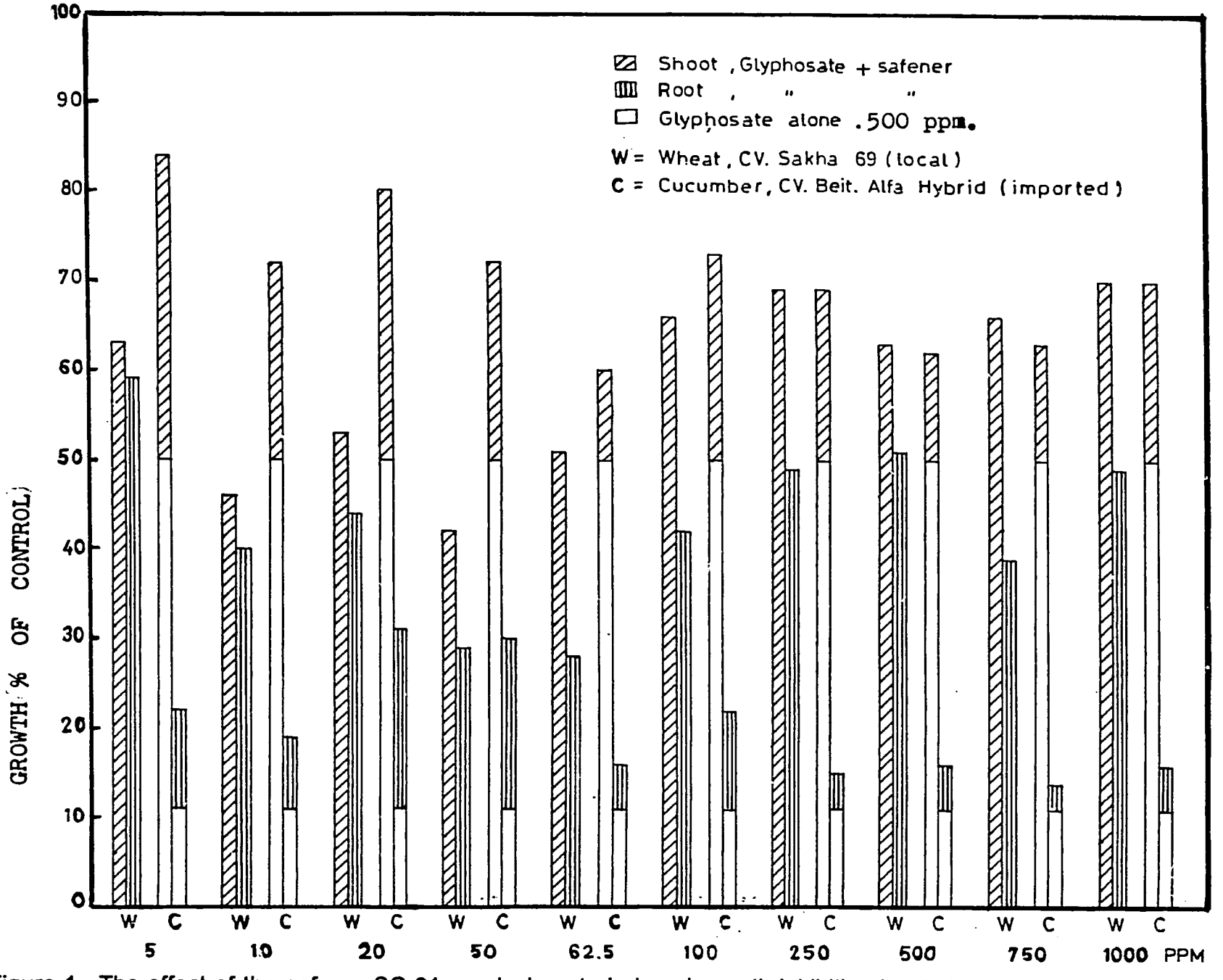


Figure 1. The effect of the safener SG 91 on glyphosate-induced growth inhibition in 15-d-old wheat (monocot) and cucumber (dicot).

Table 2. Effect of Vita 2000 seed dressing and phytokinin as soaking and spray treatments on 45-d-old pea plants.

Treatments	Root length (mm)	% of Control	% of Glyphosate	Shoot length (mm)	% of Control	% of Glyphosate
Control	351.2	100	120.1	168.6	100	127.8
Vita 2000 seed dressing	339.5	96.66	116.1	164.9	97.80	125.0
Phytokinin 2/1000	323.2	92.02	110.5	168.6	100	127.8
Glyphosate 125 ppm	292.4	83.25	100	131.9	78.23	100
+ Vita 2000 seed dressing	303.2	86.33	10.369	140	83.03	106.14
+ Phytokinin 2/1000 soaking	297.4	84.68	101.7	156.7	92.94	118.80
+ Phytokinin 2/1000 spray	301.1	85.73	103.65	149.2	88.49	113.11
	LSD (0.05) 6.9			LSD (0.05) 6.9		

Vita 2000 seed dressing treatment and phytokinin as a spray, both as a pretreatment, resulted in longer roots of peas than did glyphosate alone. Vita 2000 and phytokinin (as a soaking treatment or as a spray) resulted in higher shoot length than did glyphosate alone. Note: 1 ml/1000 of the phytokinin stock solution is equal to 150 ppm kinetin (6 furfuryl amino purine)

c. Germination bioassay laboratory test of SG 91 safener compound against butralin on cucumber (Table 3).

The laboratory bioassay test of SG 91 and butralin (48%) on 'Beit Alfa Hybrid' cucumber was carried out by soaking seeds in the herbicide (250 ppm) for 30 min followed by soaking in the safener at 100, 250, or 500 ppm for 1 h. Shoot and root responses to the treatments were recorded on 10-d-old seedlings (Table 3).

Seedling shoot growth, after seed soaking in butralin (250 ppm), reached 65.9% of the control. No additive shoot growth resulted from sequential exposure to SG 91 at 500, 250, and 100 ppm (Table 3). In the roots, sequential treatment with 100 ppm SG 91 after butralin promoted root growth over the control, i.e. 116.8% of the control. Butralin alone affected the shoot epicotyl, and side roots (Figure 2). SG 91 alone increased root growth significantly. Interaction of herbicide and safener affected side root growth, number, and distribution. Inhibitory effects on the shoot epicotyl were irreversible.

Based on the results, butralin could be used as soil-applied treatment at early stages of *Orobanche* on the roots (side roots are mostly the target of *Orobanche* establishment).

3. Host reaction to *Orobanche* infection. In field visual developmental morphology of *Orobanche* parasitism on faba bean.

Personnel: E. S. Hassan

Faba bean cv. Giza was sown on Oct. 25, 1993 in two plots naturally infested with *Orobanche* at the Experimental Station, ARC. Periodic examinations for *Orobanche* growth and development on the host roots were carried out and growth records were taken as follows:

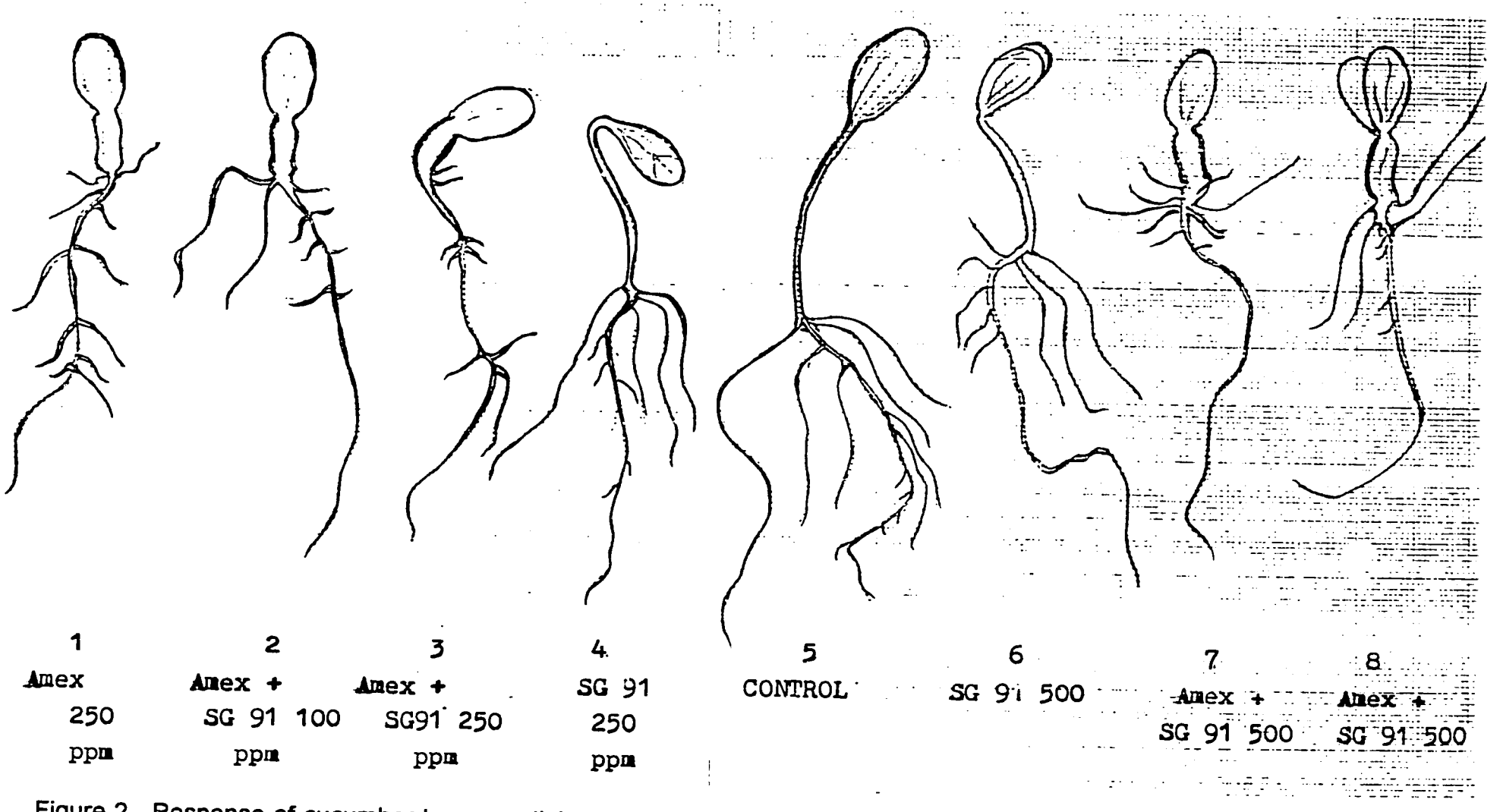


Figure 2. Response of cucumber to sequential treatments of butraiin (Amex) and the safener SG 91. Notice the irreversible effect on shoot epicotyl and change in side root number and distribution of 10-d-old cucumber seedlings.

Table 3. The effect of SG 91 safener and butralin on seedling shoot/root development of cucumber, recorded 10 d after germination.[a,b]

Treatments	Concentration (ppm)	Root			Shoot		
		Length (mm)	% of control	% of butralin	Length (mm)	% of control	% of butralin
SG 91	500	55.1	93.7	99.4	33.8	103.0	156.5
	250	45.8	77.9	82.7	23.1	70.4	106.9
	100	22.2	37.8	40.1	26.2	79.9	121.3
Butralin	250	55.4	94.2	100	21.6	65.9	100
+ SG 91	500	59.0	100.3	106.5	19.6	59.8	90.7
	250	60.2	102.4	108.7	20.8	63.4	96.3
	100	68.7	116.8	124	20.7	63.1	95.8
Control	0	58.8	100	106.1	32.8	100	151.9

Note:

[a]Average of 100 seedling measurements.
[b]Sequential application of safener compound promoted growth of roots as compared to each of the herbicide and SG 91 in single treatments.

On faba bean: - Number of fully-expanded leaves.
- Apex situation and existence of healthy floral organs on the main shoot.

On broomrape: - Developmental morphology of the vegetative and generative growth stages of the parasite was recorded.

Sum: Host reaction to growth stages of *Orobanche* was described.

Results are shown in Tables 4 and 5 and Figure 3.

The life cycle of broomrape on faba bean roots was identified as six developmental growth stages in respect to host reaction. The rhizoidal stage on the roots is the crucial stage in respect to host development. It reflects the inhibition of the floral organs' development in an acropetal order on the main shoot of the host plant. The phenomenon was terminated by the cessation of main apical activity which is irreversible, i.e. could not be restored. Such a host reaction coincided with the emergence of broomrape from soil. Complete death and deterioration of the host accompanied maximum developmental productivity of *Orobanche,* i.e. blooming and seed production.

The results indicate that *Orobanche* control should start before its crucial underground rhizoidal stage. Broomrape as a target for control should be suppressed before or at such a stage (Figure 3). The objective of the host treatments is to be based on restoring and maintaining its generative capacity against the incidence of infection, which mainly caused irreversible defloration.

4. Plans for the next season (Oct. 1994 - May 1995) at NRC.

   a. Proceed with the survey and host-*Orobanche* species registration, and measure crop losses/damage.

   b. Test for confirmation results of safeners against butralin and glyphosate on different hosts in the laboratory and greenhouse.

   c. Conduct experiments on combination of safener compounds to select for maximum efficiency with a given herbicide for *Orobanche* control.

   d. Carry out chemical analyses for detection of different hosts reaction to infection, and to control treatments.

   e. Plans at the Ministry of Agriculture are to proceed with the survey and start experiments on chemical control.

Table 4. Morphology reaction of host plant to *Orobanche* developmental stages during the growing season, 1993/1994 of 260 plants.

Age	Parasite growth stage	% Healthy apex		Number of leaves	
		Healthy	Infected	Healthy	Infected
58	establishment			9.50	9.50
68	I	100	100	11.50	11.50
92	II	100	96	16.50	16.25
105	III	100	0.0	22.75	20.15
115	IV	-	-	26.8	16.50
125	V	-	-	30.0	-
135	VI	-	-	-	-

[a]75% of the plants in free and infected plots reached the flowering stage as calculated separately for 250 plants in each plot.

Table 5. Growth measurement of *Orobanche* emerging successivly during the growth season of the host-each value is the mean of 360 *Orobanche* individuals.

Period from sowing to emergence, days	Total height (cm)	Length of generative portion (cm)	Generative portion %	Total number of capsules per *Orobanche* stalk
105-107	42.23	28.83	68.19	37.10
[illegible]	35.50	16.50	48.61	18.98
112	30.50	14.83	46.69	17.08
115	24.33	10.44	42.75	11.65
120	16.44	5.80	35.40	6.35
125	11.50	3.31	29.91	3.43

Note: Generative capacity of *Orobanche* on faba bean is season-dependent.

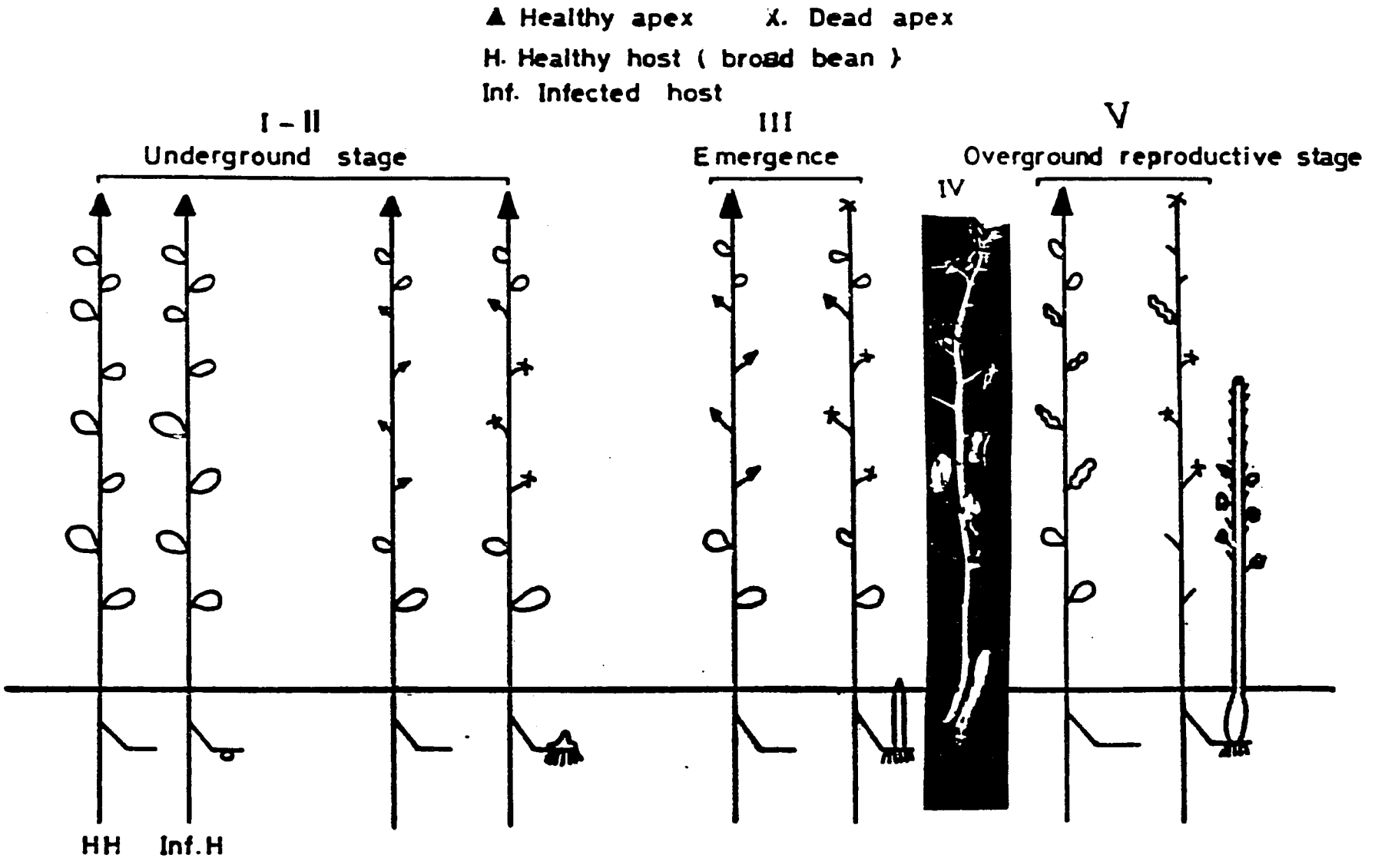


Figure 3. Illustrative drawing describes morphologically *Orobanche* growth stages and the competition between host and parasite reproductive cycles.

Israel - Reuven Jacobsohn and David Gidoni (Principal Investigators)

B. Molecular Identification of Broomrape Strains, Prediction, and Utilizing Species Phenology in Avoidance and Control.

Part 1. (R. Jacobsohn)

This progress report includes a summary of seven field experiments. We conducted most of the planned work.

1. Testing various soil mixtures for greenhouse pot experiments. Consistent with Working Plan 3.8.1.

We tested various mixtures of sand, tuff (ground volcanic stone), medium clay soil, fine vermiculite, and methylbromide-fumigated loam soil. Five mg of seeds of *O. aegyptiaca* or *O. crenata* were mixed into each liter of air-dry soil mixture. The 2-L pots were planted with tomato in the *O. aegyptiaca*-infested soil, and broadbean in the *O. crenata*-infested soil mixture. The best infestation was obtained using the mixture of two volumes of Bet Dagan clay soil and one volume of fine vermiculite.

2. Studies on broomrape host range. Consistent with Objective 2.1 and Working Plan 3.1.3.

The purpose of this study was to open options for growing crops in broomrape infested fields, as some crops can be grown in a field infested with one broomrape species but not in another. For that purpose, we established a field with plots infested with pure stands of two strains of *O. aegyptiaca, O, cumana, O. crenata, O. muteli*, and *O. cernua*. The purpose of the present study was to verify results obtained in the broomrape test plots in past years. Those experiments will be conducted in farmers' fields "naturally" infested. Attempts will be made to test the host range of each broomrape species regarding winter and summer crops.

a. *Orobanche aegyptiaca* - winter crops. Location - Kibbutz Gesher Haziv, Western Galilee.

The following 16 winter crops were tested in a field infested with *O. aegyptiaca* - summer strain: cabbage, cauliflower, chinese cabbage, rape, celery, parsley, carrot, clover, broadbean, pea, vetch, chickpea, lettuce, flax, and safflower. Each plot consisted of two, 4-m long rows. The experiment was planted on Oct. 20-21, 1993. Only pea and clover were totally free of broomrape infection.

b. *O. crenata* - winter crops. Location - Kibbutz Sde Eliyahu, Bet Shean Valley. We tested the same crops using the same experimental design. Potato replaced carrot. The experiment was planted on Oct. 26-27, 1993.

Crops that were totally free of broomrape infection were: crucifers (cabbage, cauliflower, chinese cabbage), potato, clover, and flax. Unusually low infections occurred on celery, parsley, chickpea, and lettuce. Probably, there are differences in virulence among populations within the species.

c. *O. crenata* - summer crops. Location - Kibbutz Ein-Harod in the Valley of Jezre'el. Eight summer crops were planted May 4, 1994. The experiment is currently in progress.

d. *O. muteli* - summer and winter crops. Location - Ein-Zivan - northern Golan heights (900 m above sea level). Sixteen crops were planted June 6-7, 1994. The experiment is currently in progress.

3. Prediction of broomrape potential in the field. Consistent with Objective 2.2 and Working Plan 3.2.1 and 3.2.3.

There are three components to that objective, as follows: a) quantitative isolation and determination of broomrape seeds from the soil; b) field sampling; and c) correlating levels of infestation as determined in the laboratory with crop damage.

We had to introduce a preparatory stage --- sieve the air dry soil and thoroughly disperse the sample in water by adding a few grams of Na_2CO_3 and stir the sample with an electric mixer for 15 min. Although we have reached a recovery of above 80%, we have to improve on separating the seed from organic matter of similar size, which is slowing the counting of the seeds. We have already started to work on the third component - correlating between infestation and yield loss. That is carried out by sampling soil and crop yield from several sites in infested fields. We sampled the most infested and broomrape-free spots, as well as intermediate infestations. So far, we produced data from one pea field with *O. crenata*, ($r = 0.899$), two carrot fields with *O. crenata* ($r = 0.65$ and 0.84) and one carrot field with *O. aegyptiaca* ($r = 0.83$). Also, we sampled three tomato fields but have not analyzed the soil yet. The results of the pea field sampling are presented in Figure 4.

4. Broomrape survey. Consistent with Objective 2.1 and Working Plan 3.1.1.

It is being and will be done in the following stages: a) processing existing information; b) collecting information by Dr. Jacobsohn and his team; and c) issuing a questionnaire to the farmers and extension service personnel in the form of post cards and a leaflet with colored pictures of the different broomrape species. Each infested field will be marked on maps using different colors to designate the broomrape species. Each site will be numbered and details about the infestation will be recorded separately on a computer.

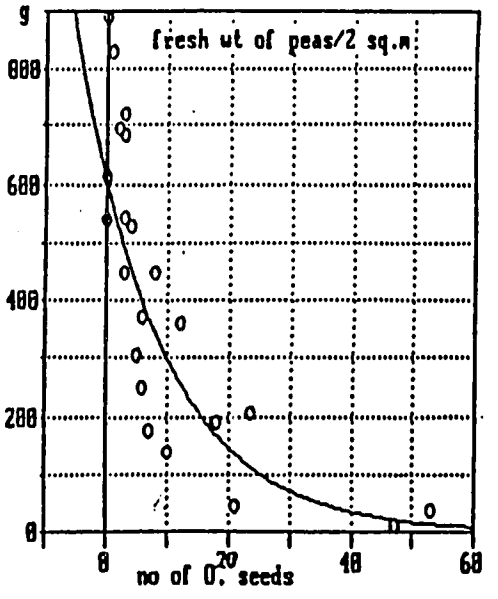


Figure 4. Fresh weight of peas and number of *O. crenata* seeds in a soil sample from a broomrape infested field.

5. Constraints.

The late arrival of the funding caused some difficulties. We will start the experiments regarding Objective 2.5 in the Fall or 1994 or Spring of 1995. We had great difficulties in acquiring the growth chamber. We invited the agent of Lab-line Instruments, Inc. in Illinois and ordered a chamber. In spite of repeated requests, we did not receive a proper answer. We plan to look for an alternative soon. In the meanwhile, a phytotron will be completed soon in the ARO, and we may rent space there.

6. Future plans.

To continue according to working plan. Host range studies: *O. aegyptiaca* - summer crops. *O. cumana* and *O. cernua*: summer and winter crops. Major efforts will be invested in Objective 2.2 - prediction of broomrape damage. Work on Objective 2.5 will also start soon.

7. Cooperation with Egypt.

Dr. Hassan visited with us July 26-27 and discussed our work. She visited three field experiments. We agreed that Dr. Jacobsohn will visit his Egyptian counterparts in January 1995 and two of the Egyptian colleagues will visit and train in the Volcani Center in the Spring of 1995.

Part 2. (D. Gidoni)

1. DNA fingerprinting of broomrape species.

Our primary activity was to establish optimal DNA extraction and RAPD reaction conditions for broomrape. We have first collected samples of three *Orobanche* species: *O. crenata* (in the fields of Kibbutz Sde Eliahu), *O. aegyptiaca* (in Kibbutz Gesher Haziv), and *O. cumana* from Bet Dagan. Both the *O. crenata* and the *O. aegyptiaca* samples were collected separately, from several different host crops. In addition, samples of *O. crenata* expressing variable phenotypes were collected in a pea field near Kibbutz Ein Harod and subgrouped according to pigment intensity. Total petal DNA from each group was extracted according to Doyle and Doyle (Focus 12:13-15, 1990) and was subjected to RAPD fingerprinting analysis according to Kangfu and Pauls (Nucl. Acids Res. 20:2606, 1993). A preliminary RAPD screening was performed using several 10-mer nucleotide primers that were recently purchased from Operon, Inc., Alameda, CA.

A representative analysis shown in Figure 5, illustrates clear polymorphic fingerprints differentiating between *O. cumana* (lane 1), *O. crenata* (lanes 2-12), and *O. aegyptiaca* (lanes 13-23). Similar results have been consistently obtained using several other primers. In addition, polymorphic patterns could also be detected: a) within a phenotypically varying *O. crenata* population that was sampled from a pea field in Ein Harod (compare lanes 2 through 5); b) within *O. crenata* grown on different hosts (data not shown). These results should be further tested and substantiated before any conclusion could be made.

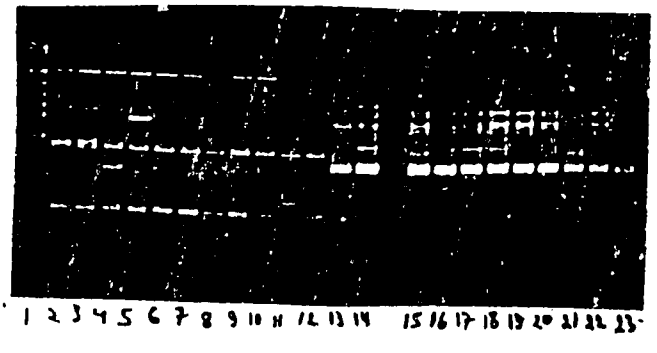


Figure 5. Agarose gel electrophoretic analysis of RAPDs of genomic DNA from *O. cumana* (lane 1), *O. crenata* (lane 2-12) and *O. aegyptiaca* (lanes 13-23), on sunflower (1), pea (2-6), lentils (7, 19), broadbean (8, 17), vetch (9), chickpea (10, 18), safflower (11, 16), carrot (12, 20), cauliflower (13), Chinese cabbage (14), cabbage (15), parsley (21), rape (22), and spinach (23). Lanes 2-5 represent fingerprints of *O. crenata* from a pea field in Ein-Harod, subgrouped according to phenotypic variations regarding pigment intensity (from white to purple) in the corolla, stigma, and stem.

We have recently collected several individual samples of *O. muteli, O. aegyptiaca*, and *O. cernua* from a tomato field in Neot Hagolan, as well as *O. cumana* from Bet Dagan. These samples will be subjected to a study of: a) intra-species polymorphism; b) polymorphism due to geographical distances; and c) genetic distances between, mainly, *O. muteli* and *O. aegyptiaca* as well as between *O. cernua* and *O. cumana*.

Consistent with these research goals, our activities for the next 6 mo will include: a) continual screening and identification of additional primers which expose polymorphism between and within broomrape species according to phenotypic variations, geographical locations, and host range; and b) testing the reproducibility and consistency of these polymorphic markers.

For the next 18-mo period, we will concentrate on identification of RAPD markers that enable the detection of specific *Orobanche* species and races. In collaboration with the Egyptian group, we will compare supposedly botanically identical species in Egypt and Israel.

2. Constraints.

The funds for research became available at Volcani in March 1994.

3. Collaboration.

In a meeting with Prof. Esmat Hassan (Bet Dagan, July 27, 1994), we discussed and agreed on the necessity of a visit of an Egyptian scientist in the Volcani laboratory in order to: a) exchange technical information, and b) set up the basis for the fulfillment of our collaborative goals. The details to this agreement will be further discussed during the forthcoming First Annual Egypt-Israel-USA Workshop in Israel (Nov. 1994).

U.S.A. - C. L. Foy (Principal Investigator)

C. Mineral Nutritional Effects on Germination, Parasitism and Host Tolerance, Integration into *Orobanche* Control.

First year objectives were to: obtain renewal of permission from PPQ, APHIS, USDA, and VDACS to work with *Orobanche* under quarantine; obtain equipment and prepare isolated work areas; collect seeds of different *Orobanche* spp. and strains; and conduct studies using nitrogen fertilizer in the presence of germination stimulants or root exudates.

Necessary permission was obtained from the USDA-APHIS and the Virginia Department of Agriculture and Consumer Service to work with *Orobanche* at Virginia Tech, under quarantine. A special air tight room was constructed in the laboratory for use in transferring seeds.

Dr. James H. Westwood was employed as a Research Associate effective May 1, 1994. Vijay Nandula, a Ph.D. level Graduate Research Assistant, also joined the project during the summer.

Despite a late start for the project in Virginia, much of the required equipment has been purchased. A growth chamber has been ordered and should arrive before the end of 1994. Electrophoresis equipment for the separation of nucleic acids has been purchased, as have the necessary supplies needed for physiological research on *Orobanche*.

Orobanche seeds have been collected. We have seeds of *O. aegyptiaca, O. ramosa, O. crenata, O. cernua,* and *O. cumana* provided by Dr. Y. Kleifeld (Israel). Seed samples have also been requested from Dr. Esmat Hassan (Egypt). In addition, Dr. Robert Eplee of the USDA *Striga* Laboratory has provided us with samples of seed from four U.S. populations of *O. minor*. These seeds were collected from sites in Thomas County, Georgia; North Augusta, South Carolina; Due West, South Carolina; and Mitchell County, North Carolina. We have also obtained a few *O. minor* seeds from an infestation in Washington County, Virginia.

1. Response of *Orobanche* seeds to germination stimulants.

Our first research priority was to test the germination rate of our seed supplies in order to optimize conditions for germination of *Orobanche* seeds in the laboratory. The germination stimulant, strigol, and its synthetic analogs GR 24 and GR 7 were applied at concentrations of 0.01, 0.1, and 1.0 mg/L to seeds held between layers of glass fiber filter paper (GFFP), following the method of Mangnus et al. (1992). The highest germination percentage was obtained with *O. aegyptiaca* using strigol and GR 24 (Figure 6). In the first test *O. ramosa* and *O. cernua* also responded well to these stimulants, and *O. ramosa* was equally sensitive to GR 7. *O. crenata* germinated at a much lower rate and was more sensitive to GR 24 than to strigol, while *O. cumana* was not stimulated by any of the compounds. This experiment was repeated with similar results. The low rate of germination of *O. crenata* and *O. cumana* could be due to either different preconditioning or germination requirements than those of the other species (all five species were given identical treatment), or by lower viability of the seeds. Further tests will be performed to increase *O. crenata* and *O. cumana* germination, and a fresh seed supply has been requested.

Similar germination tests were conducted using *O. minor* populations. Under these conditions the Virginia population exhibited a relatively high response to the GR 24 and strigol (Table 6). The other four populations, however, germinated only in very low percentages. This experiment was also repeated with similar results. Several approaches are being tried in order to increase germination in these populations (discussed below).

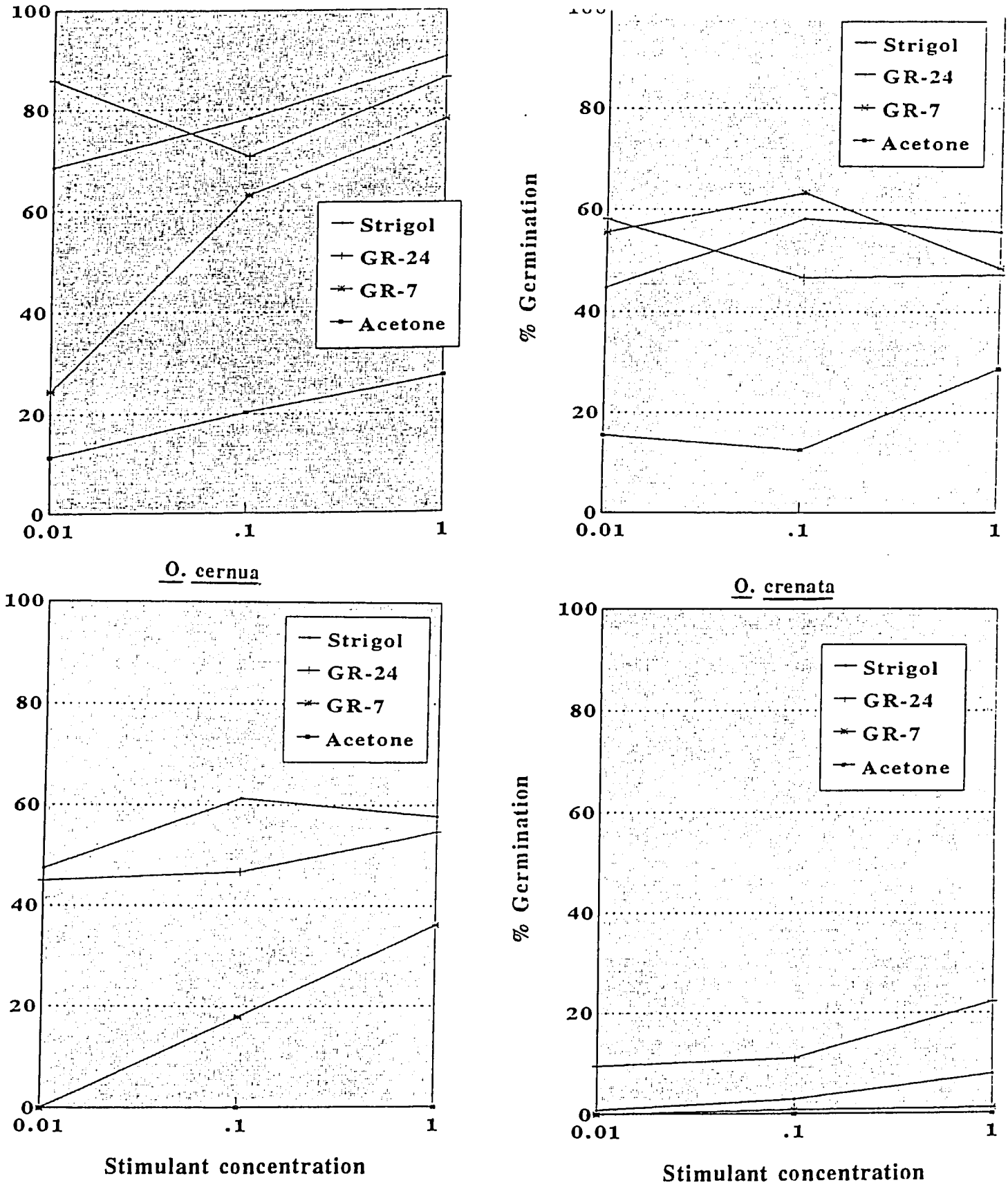


Figure 6. Germination of four *Orobanche* species after exposure to three germination stimulants. Acetone was used as a solvent in all stimulant solutions and alone represents a control.

Table 6. Germination of seeds from five U.S. *Orobanche minor* populations after exposure to 1.0 mg/L of three germination stimulants.

	% Germination				
	VA	NC	GA	North Augusta, SC	Due West, SC
Strigol	82	4	0	0	0
GR-24	83	3	0	0	0
GR-7	0	0	0	0	0
Control	0	0	0	0	0

2. Effect of nutrients on germination of *Orobanche* seeds.

The effect of nutrients on *O. aegyptiaca, O. ramosa,* and *O. minor* (Virginia) seed germination was studied using KNO_3, $(NH_4)_2SO_4$, $Ca(NO_3)_2$, K_2SO_4, KH_2PO_4, and NH_4NO_3 salts. These salts were applied in 1 M solutions to preconditioned seeds in the manner of the germination stimulant experiments described above. For all species tested the distilled water control yielded the highest germination percentage while the nutrient solutions either had no effect or decreased germination (Table 7). The lowest percentages were in treatments containing $(NH_4)_2SO_4$ and KH_2PO_4. Salts containing nitrogen in the nitrate form had little effect on germination. These results are consistent with those of Jain and Foy (1992) who found that preconditioning *O. aegyptiaca* seeds in the presence of ammonium and/or phosphate salts inhibited GR 24-induced germination.

A similar experiment examined the effect of adjuvants on germination of *O. aegyptiaca* and *O. ramosa* seeds. Preconditioned seeds were treated with adjuvants at various concentrations and rated for germination. The adjuvants used included two blends containing some organosilicones (Kinetic and Dyne-Amic), methylated soybean oil (MOS), and two pure organosilicone adjuvants (Sylgard 309 and Silwet L-77). Distilled water was used as a control. *O. aegyptiaca* germination was decreased by Kinetic and Dyne-Amic (Table 8). Although MOS, Silwet L-77, and Sylgard did not alter germination in *O. aegyptiaca*, they enhanced germination of *O. ramosa* seeds. These conflicting trends may indicate a species specific response to adjuvants which may reflect different germination signals in *Orobanche*. Questions about the effect of adjuvants on seed in the soil await further investigation.

Table 7. Effect of nutrients on germination of *Orobanche* seeds.[a]

Treatment	% Germination		
	O. aegyptiaca	*O. minor*	*O. ramosa*
KNO_3	12 cd	18 a	30 ab
$(NH_4)_2SO_4$	12 d	09 cd	10 d
$Ca(NO_3)_2$	30 bc	16 ab	21 bc
NH_4NO_3	41 ab	08 cd	32 a
K_2SO_4	19 cd	04 de	19 d
KH_2PO_4	14 cd	00 e	11 d
Water	51 a	12 bc	23 abc

[a]Means within species followed by the same letter are not different at $\alpha = 0.05$.

Table 8. Effect of adjuvants on germination of *Orobanche* seeds.[a]

Treatment	% Germination	
	O. aegyptiaca	*O. ramosa*
Kinetic	26 b	20 c
Dyne amic	34 b	23 bc
MOS	55 a	39 ab
Silwet L-77	65 a	43 a
Sylgard	69 a	52 a
Water	58 a	20 c

[a]Means within species followed by the same letter are not different at $\alpha = 0.05$.

3. *Orobanche* culture strategies.

Another research priority has been the development of methods for the growth and study of *Orobanche* in the laboratory and greenhouse.

Several strategies for growing *Orobanche* have been pursued with the objective of obtaining material for physiological and biochemical studies of the parasite and host-parasite interactions. The first method was to simply grow *Orobanche* on host plants. Host plants used in this work included red clover, carrot, cowpea, sunflower, and tomato. All *Orobanche* species and populations were applied to the bare roots of the appropriate host plant seedlings at the time of transplanting. The first trial used commerical greenhouse potting soil which was not conducive to growth of the parasite. Attachment and development of *Orobanche* was very sporadic. Subsequent trials have used a mix of sand, topsoil, and perlite (1:1:1 by volume). This has been much more effective in allowing parasite attachment to hosts and development to the point of emergence has occurred within 7 wk of inoculation.

An alternative method for establishing *Orobanche* on host plants is the glass plate sandwich method (Jacobsohn et al., 1990) which allows viewing of the attachment process. This has been tried in order to find an efficient method of ensuring that host plants are infected with *Orobanche*. At this point the method works well for a few weeks but the host plants have been subject to fungal infections. After parasite attachment has occurred, host plants may be transplanted into soil mix or may be placed into hydroponic culture for continued study of parasite development.

Tissue culture of *Orobanche* has also been tried in order to produce plant material and as a system to investigate parasite physiology. First, a method for surface sterilizing *Orobanche* seed was developed. Best results were obtained with a 20-s exposure to 70% ethanol followed by 30% bleach (Chlorox) with 1% Triton X-100, agitating for 20 min. Sterilized seeds were plated on agar medium (Ben-Hod et al., 1991) and stored in the dark. *Orobanche* species tested were *O. aegyptiaca, O. ramosa,* and all *O. minor* populations. Germination rate was high for *O. aegyptiaca, O. ramosa,* and *O. minor* (Virginia). The other *O. minor* populations had low germination rates (similar to those of the germination study). Seeds that germinated were transferred to maintenance medium and have continued to proliferate.

Due to difficulties in germinating seeds of the *O. minor* populations, some alternative germination media were tried in order to establish cultures of these populations. The original germination medium contained 20 mg/L GA_3. Variations on this included the addition of strigol (1 mg/L), NAA (0.1 mg/L), and increasing GA_3 concentration to 50 mg/L. At this time neither of these media has produced greater germination than the original medium.

Although the *O. minor* populations have a very low germination rate in response to strigol, they germinate to a greater extent when in the presence of a host plant or exudates collected from a host plant. A preliminary experiment with clover, carrot, soybean, and sunflower indicated a stimulatory effect on seeds when exposed to a nutrient solution in which host roots had been bathed for several days. The use of host specific stimulants is being used to maximize germination in the *O. minor* seeds.

4. Future work.

a. Six-months plan.

Investigations into the effect of nutrients on *Orobanche* germination will be continued. Experiments have already been started in the greenhouse to further define the role of nitrogen fertilizers in germination, growth, and development of *Orobanche*.

O. minor populations in the U.S. will be characterized. We have assembled the equipment and chemicals needed to perform PCR/RAPD analysis on the U.S. populations of *O. minor*. At this time we are only waiting to obtain tissue from which to extract DNA. This technique is a powerful tool for distinguishing variation within species. Very little work has been done on the population biology of *Orobanche* and these U.S. populations will provide an interesting set of material for comparisons. PCR/RAPD characterization will enable us to make inferences about the genetic variability of these populations and speculate on their origins. Comparisons between infestations will permit speculation about whether they resulted from independent invasion events or if there was spread from site to site within the U.S. Also, further work testing various host root exudates on the different populations may indicate differences in host preference between populations. This will result in both genetic and physiological characterization of these populations.

Host plants are being grown in order to study morpho-anatomical features of the host-parasite interface and haustorium connnections.

One of the most promising strategies for the control of *Orobanche* is the engineering of herbicide resistance into host crops such that a systemic herbicide applied to the crop will translocate harmlessly through the host to the parasite and halt its growth before it can significantly injure the host. We have soybean seed (Asgrow A4045) that was selected for resistance to the sulfonylurea herbicides and are in the process of attempting to parasitize seedlings of this line with *Orobanche*. Our objective is to study the processes of haustorial attachment and development in the presence of herbicide translocated from the host. *O. crenata* has been shown to attack soybean (Jain and Foy, 1989) and is being used in these studies.

Studies are currently underway employing transgenic soybeans having the phosphinothricin acetyltransferase (PAT) resistance gene which reportedly confers

resistance to the herbicide glufosinate (Ignite). Attempts to obtain transgenic plants and/or gene constructs for incorporating herbicide resistance into important crop species will be continued.

b. Eighteen-months plan.

Aspects of nitrogen metabolism of *Orobanche* will be investigated. In relation to the work on the effect of nitrogen on *Orobanche* germination, studies will investigate the ability of *Orobanche* to metabolize nitrogen and nitrogenous compounds. Very little is known about the nitrogen metabolism ability of *Orobanche*, yet the procurement of nitrogen is one of the primary goals of parasitism. We will study amino acid profiles of the parasites and their hosts as well as conduct further investigations into the nitrogen assimilation ability and protein content of the parasites. In conjunction with this, tissue culture of *Orobanche* will be essential in making comparisons between *Orobanche* growing in association with a host and that living solely on a nutrient medium. For this latter aspect, it will be useful to have *Orobanche* in similar developmental stages as that found on hosts so attempts will be made to regenerate shoot tissue from callus in culture by altering the hormone ratios in the growth media.

References

Ben-Hod, G., D. Losner, D. M. Joel, and A. M. Mayer. 1991. In vitro culture of *Orobanche aegyptiaca*. Ann. Bot. 68:413-416.

Jacobsohn, R., C. L., Foy, and K. Marton. 1990. Growing broomrape (*Orobanche* spp.) in a soilless system. Weed Technol. 4:804-807.

Jain, R. and C. L. Foy. 1989. Broomrapes (*Orobanche* spp.): A potential threat to U.S. broadleaf crops. Weed Technol. 3:608-614.

Jain, R. and C. L. Foy. 1992. Nutrient effects on parasitism and germination of Egyptian broomrape (*Orobanche aegyptiaca*). Weed Technol. 6:269-275.

Mangnus, E. M., P. L. A. Stommen, and B. Zwanenburg. 1992. J. Plant Growth Regul. 11:91-98.

Activity II. **Integrated Agronomic Procedures Based on Parasite/Crop Interactions**

Egypt - F. Maklad and E. A. Hassan (Principal Investigators)

A. Use of Crop Exudates to Promote Premature Parasite Germination and Prevent Formation of Germination Stimulants by Host Crops.

1. Experimental progress.

Our objective in changing sowing dates of *Orobanche* hosts was to block the synchronization between the two life cycles which resulted in the success of the parasite at the expense of the host, as the parasite depends on the life cycle of the host in response to its root exudates.

The change in sowing date coincided with exposure of the host to relatively unfavorable environmental conditions, and probably root exudates could change. However, its growth and yield is expected to be reduced. We tried to combine the change in sowing date with an application of a growth promoting substance, a commercial phytokinin, to restore normal growth of the host while (maybe) preventing *Orobanche* germination.

Healthy 'Giza 402' faba bean was planted in old soil at the NRC Experiment Station. Six sowing dates from Oct. 15 to Dec. 14 (Table 9), each combined with one or two phytokinin spray applications 1 mo and 45 d after cultivation, were employed. A concentration of 2/1000 i.e. 300 ppm kinetin (6 furfuryl amino purine) was applied. 'Lincoln' peas planted in infected soil at the Ministry of Agriculture were cultivated Oct. 15, 1993, and Jan. 5, 1994. Growth parameters, yield and infestation with *Orobanche*, were recorded on 3-mo-old host plants (Table 10).

Growth parameters of faba bean plants differed in their response to delayed sowing date (Table 9). Plant height was reduced proportionally to the delay in sowing. Number of pods per plant increased slightly up to the 3rd sowing on Nov. 8, 1993, then declined. Branching increased up to the 4th sowing date Nov. 20, 1993, then decreased. Weight of pods, weight of seeds, and weight of straw per plant increased to the 2nd sowing date Oct. 27, 1993. In combination with the phytokinin spray treatment, sowing on Nov. 20 combined with two sprays gave the highest yield.

Table 9. Effect of changing sowing date combined with phytokinin for foliar application treatments on growth and yield of faba bean. (Average of 300 plants).

Sowing date	Treatments	Plant height (cm)	No. branches/ plant	No. pods/ plant	Straw weight/ plant (gm)	Pod weight/ plant (gm)	Seed weight/ plant (gm)	Seed weight (kg/fed)
Oct. 15	Control	147.2	4.0	26.9	89.8	52.0	39.1	697.5
	One spray	143.4	4.3	36.5	98.0	85.2	67.9	868.0
	Two sprays	144.6	4.2	38.3	119.5	79.7	64.8	914.0
Oct. 27	Control	149.3	4.3	37.6	110.4	65.7	48.3	1007.5
	One spray	136.2	4.2	47.4	97.5	71.7	56.7	1069.5
	Two sprays	119.5	5.1	69.3	130.4	116.6	84.5	1100.5
Nov. 8	Control	134.6	4.6	37.6	85.6	67.8	52.0	992.0
	One spray	131.6	4.5	44.7	98.9	86.6	65.7	1069.5
	Two sprays	117.6	4.8	73.7	125.1	99.3	68.7	1209.0
Nov. 20	Control	112.5	4.7	27.5	103.6	74.4	57.2	1007.5
	One spray	110.8	4.8	41.2	85.5	88.9	69.5	1038.5
	Two sprays	110.0	4.9	45.7	108.6	80.7	62.4	1240.0
Dec. 2	Control	111.0	3.5	31.8	83.1	69.2	53.2	821.5
	One spray	96.8	3.4	27.2	79.8	71.1	51.1	1007.5
	Two sprays	98.7	4.2	35.8	75.6	67.0	50.8	1116.0
Dec. 14	Control	75.8	4.3	20.6	56.1	44.3	32.8	573.5
	One spray	79.9	4.0	27.2	62.7	47.8	36.7	790.5
	Two sprays	86.6	3.8	22.2	50.1	51.6	39.3	775.0

Table 10. Effect of two sowing dates on growth and yield of peas under *Orobanche* infestation - recorded 3 mo after sowing.

	Root length (cm)			Shoot length (cm)			No. leaves/plant			No. pods/plant			No. seeds/plant		
	Oct. 15		Jan. 5	Oct. 15		Jan. 5	Oct. 15		Jan. 5	Oct. 15		Jan. 5	Oct. 15		Jan. 5
	Healthy	Inf.	Late sowing	Healthy	Inf.	Late sowing	Healthy	Inf.	Late sowing	Healthy	Inf.	Late sowing	Healthy	Inf.	Late sowing
Mean	23.7	19.4	10.2	86.3	85.5	28.3	34.8	23.3	12.7	9.0	3.4	2.0	4.4	2.43	2.7
Reduction (%)		18.14	56.96		zero to 17	67.2		33.04	63.5		62.2	77.77		44.77	3.86
Range	12-28	11-24	7-15	45-90	37-114	23-35	14-83	8-44	10-15	1-30	3-11	1-5	3-8	3-52	1-4

Table 11. Severity of *Orobanche* recorded on peas planted during the first sowing (Oct. 15, 1993) - Area of experiment = 16 m^2.

No.	Parameters	Count/16 m^2
1	No. of host plant/area	200
2	No. of infected plant/area	172
3	No. of uninfected plant/area	28
4	No. of *Orobanche*/area	273
5	No. of *Orobanche*/plant	1-9

The loss in plant growth and yield of peas in the present experiment, for the late sowing date exceeded that in the infected ones, i.e. root and stem length, total number of leaves per plant and number of pods and seeds per pod/per plant (Table 10). The great variation in individual means of each plant measurement (Range, Table 10) directed the attention to the use of an improper source of seeds for cultivation, i.e. stock saved by the farmer from the previous season which showed and proved the deterioration of its characters.

The severity of *Orobanche* infection on peas planted during the first sowing date is shown in Table 11. The absence of infection (date not shown) completely in the late sowing date proved our hypotheses of escaping infection.

2. Plans for the next season.

a. Experiments on sowing date spectrum will be carried out on peas with a standard source of local seeds and with imported material from seed companies.

b. The change of sowing date will be combined with the main shoot decapitation and growth promoting factor's application, to affect the root stimulant.

c. Intercropping with nonhost and atypical hosts will be conducted, to use root stimulants for blocking life cycle synchrony of the desired crop with the existing broomrape spp.

d. Chemical analysis.

Israel - Y. Kleifeld (Principal Investigator) with **Y. Goldwasser** and **D. Plakhine**

The research in 1993/94 was based on results obtained from an intensive split field trial which was reported earlier (Kleifeld et al., 1994) (while this project was being evaluated). From these results one may conclude that in a field heavily infested with *Orobanche aegyptiaca*, the use of trap crops in rotation, practically failed in reducing the damage to host crops. Furthermore, crops like flax (*Linum usitatissimum*), clover (*Trifolium alexandrinum*) and mung beans (*Phaseolus aureus*) which have been mentioned in the literature as trap crops, were very effective host plants for the parasite, which flowered and produced seeds.

In 1993/94 we studied two species as trap crops for *O. aegyptiaca*: vetch in the winter and pepper in the summer. The local vetch (*Vicia sativa*) var. Sadot was developed from a cross with *Vicia purpurea* and was not infested by *O. aegyptiaca* and *O. crenata* in infested fields. In vitro, we found that vetch stimulates 58% of maximum seed germination stimulated by GR 24, but is not parasitized. Another local vetch

variety "Yovel" (belonging to *V. sativa*) stimulated only 38% of maximum seed germination but was heavily parasitized.

Field studies with pepper (*Capsicum annum*), indicated that there are broomrape tolerant varieties. In pot studies in which soil was artificially contaminated with *O. aegyptiaca* seeds, we found that different varieties demonstrated different levels of tolerance to the parasite. Repeating the pot experiment in winter conditions showed a decrease in degree of the pepper tolerance to *O. aegyptiaca* but the tolerance to (*Orobanche cernua*) was not affected (Table 12).

In vitro studies showed that pepper (var. Odem, ma'or and Shalhevet) stimulate up to eight-fold more *O. aegyptiaca* and *O. cernua* seeds in comparison to tomato (var. M-82), but Ma'or and Odem exhibited high tolerance to *O. aegyptiaca* and were totally resistant to *O. cernua* (Table 13).

1. Soil fumigants.

In studies aimed at substituting the soil fumigation with methyl bromide, for more environmentally acceptable and less expensive compounds, we screened several pesticides and concentrated our work on metham-sodium (MS). In previous work with MS we found that the main obstacle causing inefficient control of *Orobanche* is the escape of the fumigant from the soil (Goldwasser et al., 1994). In pot studies, soil mulching with polyethylene (PE) soon after MS application dramatically improved broomrape control (Table 14).

In further pot studies we found that half of the recommended MS rate was sufficient for broomrape control in soil layers deeper than 5 cm. Split application of MS did not improve *Orobanche* control in pot and field studies. Control was also not improved when MS was applied by chemigation through a dense drip irrigation system in the field.

2. Integrated control.

In studies in a naturally *O. aegyptiaca* infested field, vetch (var. "Yovel") was planted in the autumn. Low rates of glyphosate were split applied during the winter to control *O. aegyptiaca* parasitizing the catch crop. After vetch harvest, MS was chemigated by sprinkler irrigation during a fallow period. Eggplants were planted in the following spring. The catch crop and the fumigant, as well as their combined effect, reduced broomrape infestation on eggplants (Table 15).

3. Constraints.

Money did not enter our accounts into well after inception of the project and we are not given credit by our institution. After finishing the first year, we are not able to buy and pay our accounts.

Table 12. Response of peppers and tomato to *Orobanche*[a].

Season[b]	17/5-11/8			7/7-31/8			3/10-23/3			3/10-23/3		
Host	Fresh weight[c] (%)	Parasite/host Infl.	Parasite/host Total	Fresh weight[c] (%)	Parasite/host Infl.	Parasite/host Total	Fresh weight[c] (%)	Parasite/host Infl.	Parasite/host Total	Fresh weight[c] (%)	Parasite/host Infl.	Parasite/host Total
Peppers												
Odem	217	0.1	1.8	192	1.7	9.5	12	2.0	8.5	81.6	0	10.0
Ma'or	165	0	0.1	127	2.9	11.5	68	1.7	20.0	74.3	0	0
Bikura	201	0.2	1.6	180	0.9	8.2	55	0.6	13.4	79.4	0	0
Orly	110	1.0	3.6	74	3.3	25.7	38	2.0	8.8	86.8	3.0	3.0
Lehava	109	2.2	21.9	130	3.5	26.5	20	2.4	16.2	69.5	0	0
Tomato												
M-82	48	27.8	>100	---	10.8	19.3	-	-	-	-	-	-

[a]Transplanted to potted soil, with or without *Orobanche* infestation.
[b]Transplanting and harvesting dates.
[c]Foliage fresh weight: % of *Orobanche* infested plants from noninfested.

Table 13. The response of *Orobanche* In vitro to peppers and tomato[a].

Host	*Orobanche aegyptiaca* Seed = germ. (%) 14 DAP	Parasites/host plant 14 DAP	21 DAP	23 DAP	Inflor. 49 DAP	Total 49 DAP	*Orobanche cernua* Seed = germ. (%) 14 DAP	Parasites/host plant 14 DAP	21 DAP	28 DAP	Inflor. 49 DAP	Total 49 DAP
Pepper												
Odem	44.8	0.2	3.7	13.4	7.0	14.5	26.0	0	0	0	0	0
Ma'or	54.0	1.2	9.8	16.5	2.2	12.5	22.5	0	0	0	0	0
Shalhevet	55.8	3.7	21.5	38.4	12.7	36.7	22.8	0	0	0	0	0
Tomato												
M-82	7.2	16.2	36.2	61.6	22.0	68.5	9.5	3.5	21.8	45.9	11.0	53.3

[a]Transplanted to polyethylene bags containing *Orobanche* seeds on glass-fiber paper in nutrient solution.

Table 14. Response of *O. aegyptiaca* parasitizing tomatoes to soil fumigation with MS followed by polyethylene mulching (Pot experiment)[a].

MS[b] (kg ai/ha)	Total parasites per host plant[c] Hours between fumigation and mulching 0	18	42	No mulching
320	0	7.2	12.6	15.8
640	0	0	9.6	10.8
960	0	0	2.4	11.2
Untreated				18.4

[a]The potted soil was artificially contamined with *O. aegyptiaca* seeds.
[b]Applied by chemigation in 1000 m^3/ha water drip irrigation to dry soil.
[c]Tomatoes transplanted 20 days after fumigation and assayed 45 days later.

Table 15. *Orobanche* control in eggplants with MS following vetch[a] as a catch crop; Field experiment, Gesher Ha'ziv.

Glyphosate[b] (kg ai/ha) a	b	c	MS[c]	*Orobanche* Flowering plants/20 eggplants 50 DAP	60 DAP	70 DAP	80 DAP
0.18			+	0	0.2	1.0	2.0
0.18	0.18		+	0.1	0.3	0.6	2.6
0.18	0.18	0.18	+	0.2	0.5	1.5	2.0
0.18		0.18	+	0.1	0.5	1.1	1.1
Control			+	1.0	2.8	6.2	9.7
Control			-	13.0	43.0	60.6	113.6

[a]Vetch Var. "Yovel", planted December 25 on raised beds.
[b]Sprayed on vetch foliage: a = January 10, on plants 8 cm high; b = January 23, on plants 12 cm high; c = March 2, on plants 20 cm high.
[c]320 kg a.i./ha, by sprinkler chemigation in 700 $m^{3/ha}$ (-) = without MS.

4. Communications.

Prof. E. Hassan visited our experiment facilities and sites in July 1994. Unfortunately we did not have the opportunity to meet our other Egyptian counterparts to coordinate our mutual work.

5. Added personnel.

Dr. D. Plakhine joined our staff and she is responsible for the laboratory work.

6. Requested addition.

We wish to include in our integrated approach the use of selective herbicides. We have recently achieved good results with sulfonylureas. Such treatment may reduce the needed rates of soil sterilants that we used in the past and result in cheaper treatments. The use of selective herbicides on catch crops and broomrape hosts fits our approach about using a crop rotation in infested fields.

7. Work plan.

Experimentation will be in accordance with the original proposal. The only point that we are not able yet to decide, is about collaboration with our Egyptian counterpart; we hope to discuss and decide about it in the coming meeting in October-November.

References

Goldwasser, Y., Y. Kleifeld, S. Golan, A. Bargutti, and B. Rubin. 1994. Metham sodium's dissipation from soil and it's effect on the control of Egyptian broomrape (*Orobanche aegyptiaca* Pers.). In: Biology and Management of *Orobanche*, Proc. 3rd Int. Workshop on *Orobanche* and Related *Striga* Research. A. H. Pieterse, J. A. C. Verkleij, and S. J. ter Borg (eds.). Amsterdam, The Netherlands, Royal Tropical Institute (in press).

Kleifeld, Y., Y. Goldwasser, G. Herzlinger, D. M. Joel, S. Golan, and D. Kahana. 1994. The effect of flax (*Linum usitatissimum* L.) and other crops as trap and catch crops for control of Egyptian broomrape (*Orobanche aegyptiaca* Pers.). Weed Res. 34:37-44.

Activity III. **Microorganisms for Control of Parasitic Weeds**

Egypt - M. Abdel-Kader (Principal Investigator)

A. Isolation of Potential Mycoherbicidal Organisms and Development of Formulation Technologies.

1. Fungi screening in Egyptian soil. Work plan section 1, timetable 1st year screening.

Our aim was to identify the naturally existing bioagents in Egyptian soil under infestation. We chose five governorates of Egypt (El Gharbia, El Dakahlia, El-Beheira, Banysweif, and Assiut) to represent Delta, Middle and Upper Egypt, because of the existence of natural infestation, different hosts, and different *Orobanche* species.

Samples were collected every two weeks from the upper mentioned governorates from two locations, one infected with *Orobanche*, and one not infected. The screening work was to select pathogen(s) for pathogenicity tests on *Orobanche* in vitro (early stages) with and without synergists (2nd year timetable). Afterward, we will proceed in the study of weakening the defense mechanism of the parasite against the bioagent for control in cooperation with the Weizmann Group. The efficiency of results will be tested under Egypt conditions. Other fauna, e.g. mites, attacked *Orobanche* and soil samples were also screened, (see Appendix).

a. Screening in Banysweif - high infestation in broadbean and chamomile.

Broadbean and chamomile fields in Somosta and Beba Centers in Banysweif governorate (Middle Egypt) were subjected to screening. Samples were collected each two weeks starting October 1993 to June 1994 from infected and noninfected locations. The marked sampling locations were cultivated with the two hosts in winter (October 1993-April 1994) and were followed by maize cultivation in summer. Collected samples were subjected to fungi screening processes, fungi isolation, identification, and relative counting intensity of existence. Pure fungal cultures were preserved on potato dextrose agar (PDA) slants at 5°C. Fungal colonies appeared on Martin's medium and were classified and examined under the microscope for accurate genera identification.

Results are shown in Figures 7 and 8. Quantitative and qualitative differences in fungi were recorded in the rhizospheres of hosts under healthy and infected conditions. Genera identified in plants' rhizospheres were *Alternaria, Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus, Phycomycetes,* and *Trichoderma.*

Total rhizosphere fungi counting increased with the increase in growth of the plants. Domination of *Fusarium, Aspergillus*, and *Penicillium* spp., respectively were detected from fall (October 1993) to spring (April 1994). During the summer, after host removal,

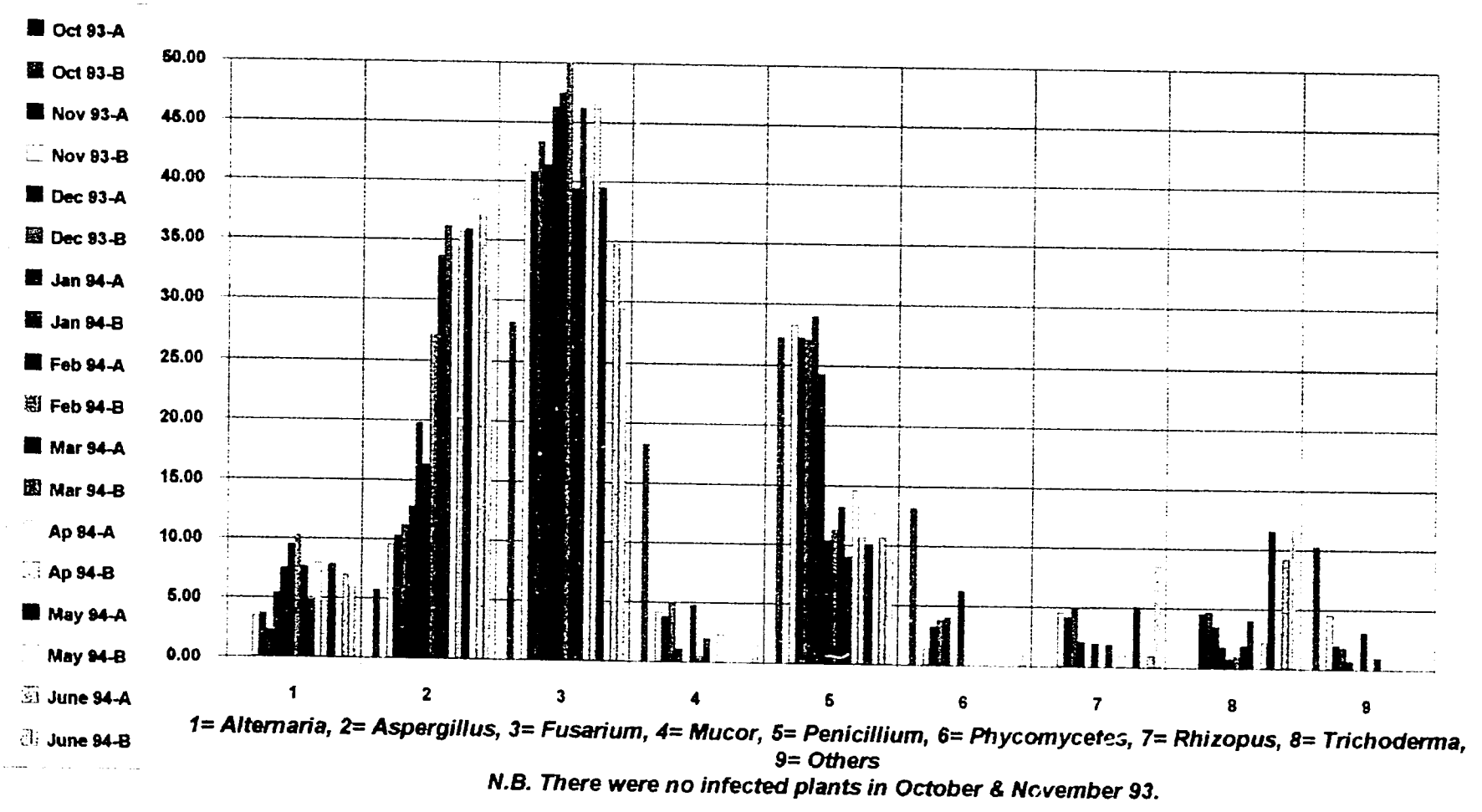


Figure 7. Percentage of occurrence of fungi in the rhizosphere of [A] broadbean plants infected with *Orobanche* sp. and [B] healthy broadbean plants during different periods of growth.

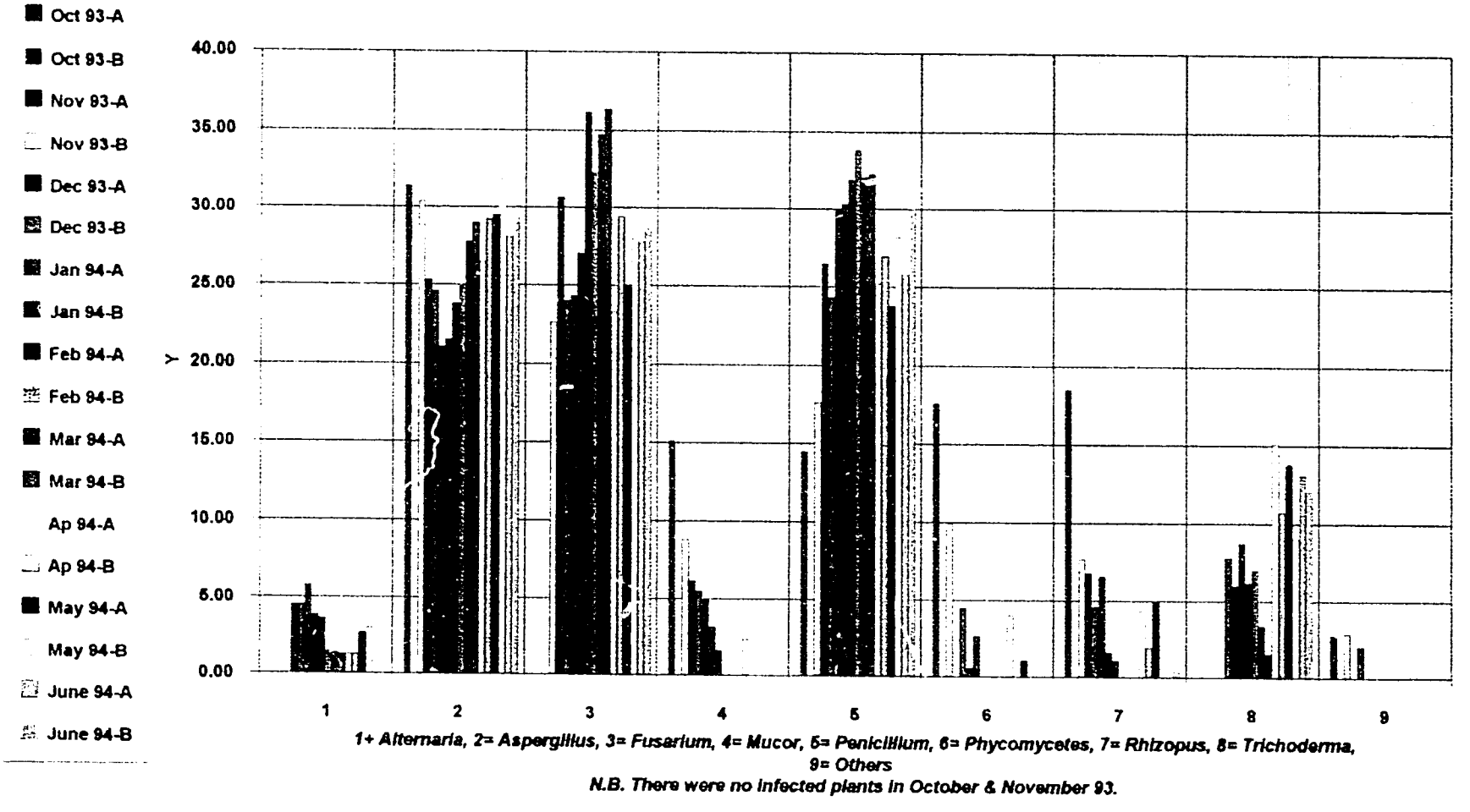


Figure 8. Percentage of occurrence of fungi in the rhizosphere of [A] camomile plants infected with *Orobanche* sp. and [B] healthy camomile plants during different periods of growth.

the trend was sustained to June 1994 in the maize rhizosphere. *Alternaria* and *Trichoderma* spp. appeared in winter (December 1993) in rhizosphere samples of healthy and infected plants and proceeded concomitantly with *Orobanche* emergence above the soil. Identification records of *Mucor, Alternaria, Trichoderma, Rhizopus* and others of unknown and phycomycetes class in rhizosphere samples fluctuated along the whole season.

b. Screening in 5 governorates.

Sampling and screening methods were the same as in a. above. Results are presented in Table 16 and Figures 9 and 10. Twelve genera of fungi were identified. *Fusarium, Penicillium*, and *Aspergillus* dominated in healthy host rhizospheres and under *Orobanche* infestation conditions, respectively, from January to March. With advancement in the season from April to June, *Aspergillus, Penicillium*, and *Trichoderma* dominated, respectively; *Fusarium* declined. The percentage of a given genus in the rhizosphere samples differed according to the governorate (Figures 9 and 10). Each genus differed in its relative count percentage in root rhizospheres of healthy from that of *Orobanche*-infected host plants (Table 16).

The represented data revealed that the existence and percentage of a given fungus in the rhizosphere is correlated with host, location, season, and *Orobanche* infestation (Table 16, Figures 9 and 10).

Israel - J. Gressel (Principal Investigator) and **Z. Amsellem** (Weizmann Institute of Science), with the cooperation of **Y. Kleifeld** and colleagues at Neve Yaar

B. Use of Synergized Mycoherbicidal Organisms to Control *Orobanche* Species.

We had planned to immediately start to study the defense responses of broomrapes against known mycoherbicidal pathogens. The putative pathogens described in the literature either did not exist any longer, or promises of availability were not kept. This set us behind at least 1.5 yr, as we had to set up to isolate mycoherbicidal pathogens by ourselves. Thus, this year's research was focused on two key issues: (1) isolation of putative primary and secondary pathogens of *Orobanche*, and (2) development of an in vitro system for testing whether isolated organisms were primary or facilitating secondary pathogens (or just "there"). Such facilitating microorganisms can act as synergists by themselves.

1. Isolation of fungi.

a. Source of isolates. Heavily infested fields were carefully scrutinized for places where the hosts looked healthiest despite the infestation, and where the broomrape was the smallest or where the emerged broomrape clearly appeared diseased. Hosts in these areas were dug and the *Orobanche* was inspected for

Table 16. Summary of fungi screening in five governorates in Egypt.

Map location	Governorate/ rhizosphere sample locations	Highest fungi genera counting		Rhizosphere under test	
		Jan.-March	April-June	Jan.-March	April-June
Delta	**El-Gharbia**				
	Infected (A)	Fusarium + Penicillium	Penicillium + Aspergillus	Cauliflower/ cabbage	Tomato Tomato
	Healthy (B)	Fusarium	Trichoderma		
	Name:M. El-Brollosy				
	El-Dakahlia				
	Infected (A)	Fusarium	Trichoderma	Faba bean	Soil/corn
	Healthy (B)	Fusarium	Aspergillus	Faba bean	Soil/corn
	Name:A. Salem				
North	**El-Beheira**				
	Infected (A)	Fusarium	Aspergillus	Peas/soil/onion	Onion
	Healthy (B)	Fusarium	Aspergillus	Peas	Potato/soil/cowpea
	Name:F. Maklad				
Middle Egypt	**Banyswelf**				
	Infected (A)	Penicillium	----------	Geranium	Geranium
	Healthy (B)	Aspergillus	----------	Geranium	Geranium
	Name:M. El-Bagoury				
Upper Egypt	**Assuit**				
	Infected (A)	Penicillium	Aspergillus	Faba bean	Faba bean/soil
	Healthy (B)	Penicillium	Aspergillus	Faba bean	Faba bean/soil
	Name:A. Korashy	Fusarium Rhizopus			

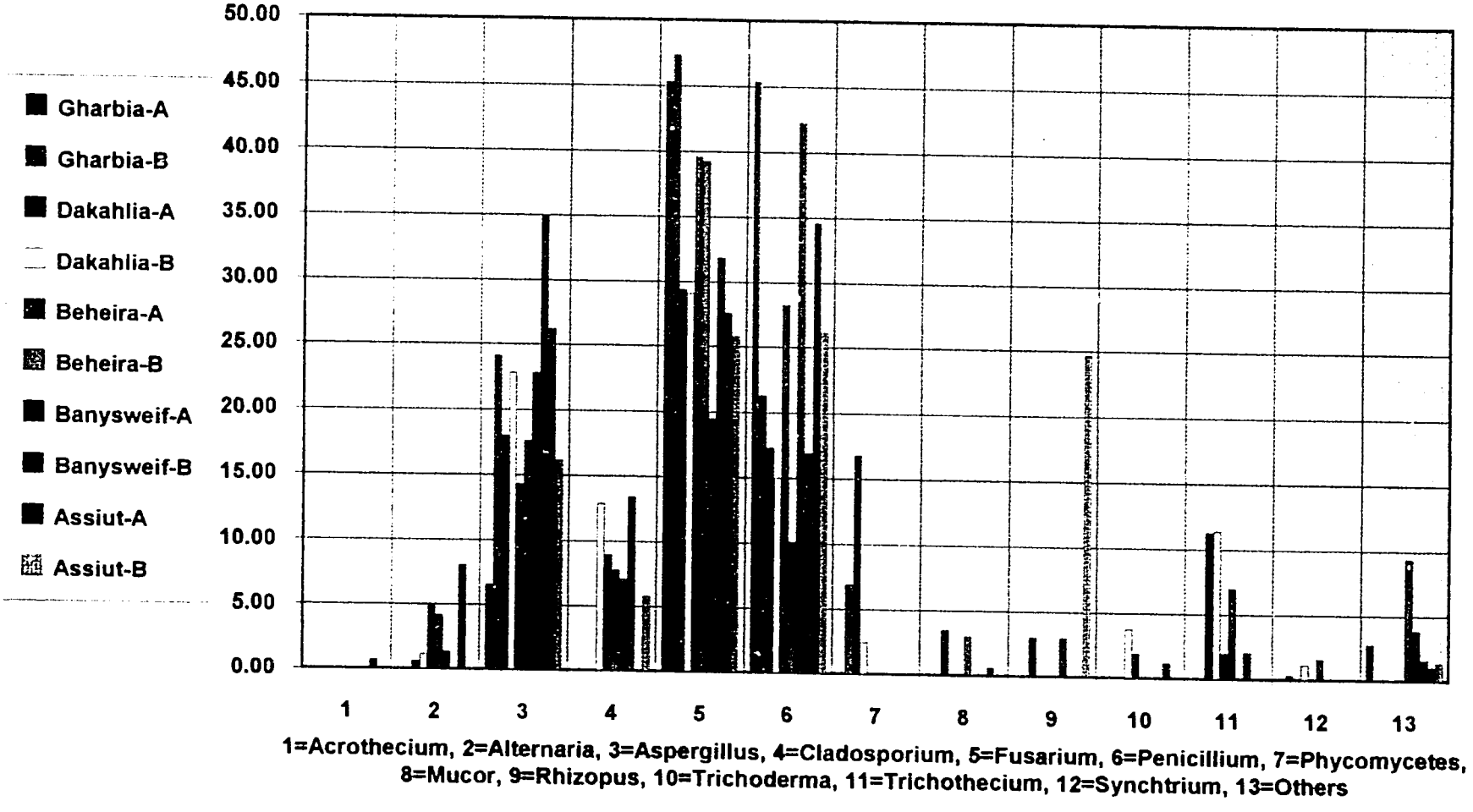


Figure 9. Frequency of occurrence of fungi in the rhizosphere of [A] plants infected with *Orobanche* spp. and [B] healthy plants in different governorates in Egypt during the period extending from January to March 1994.

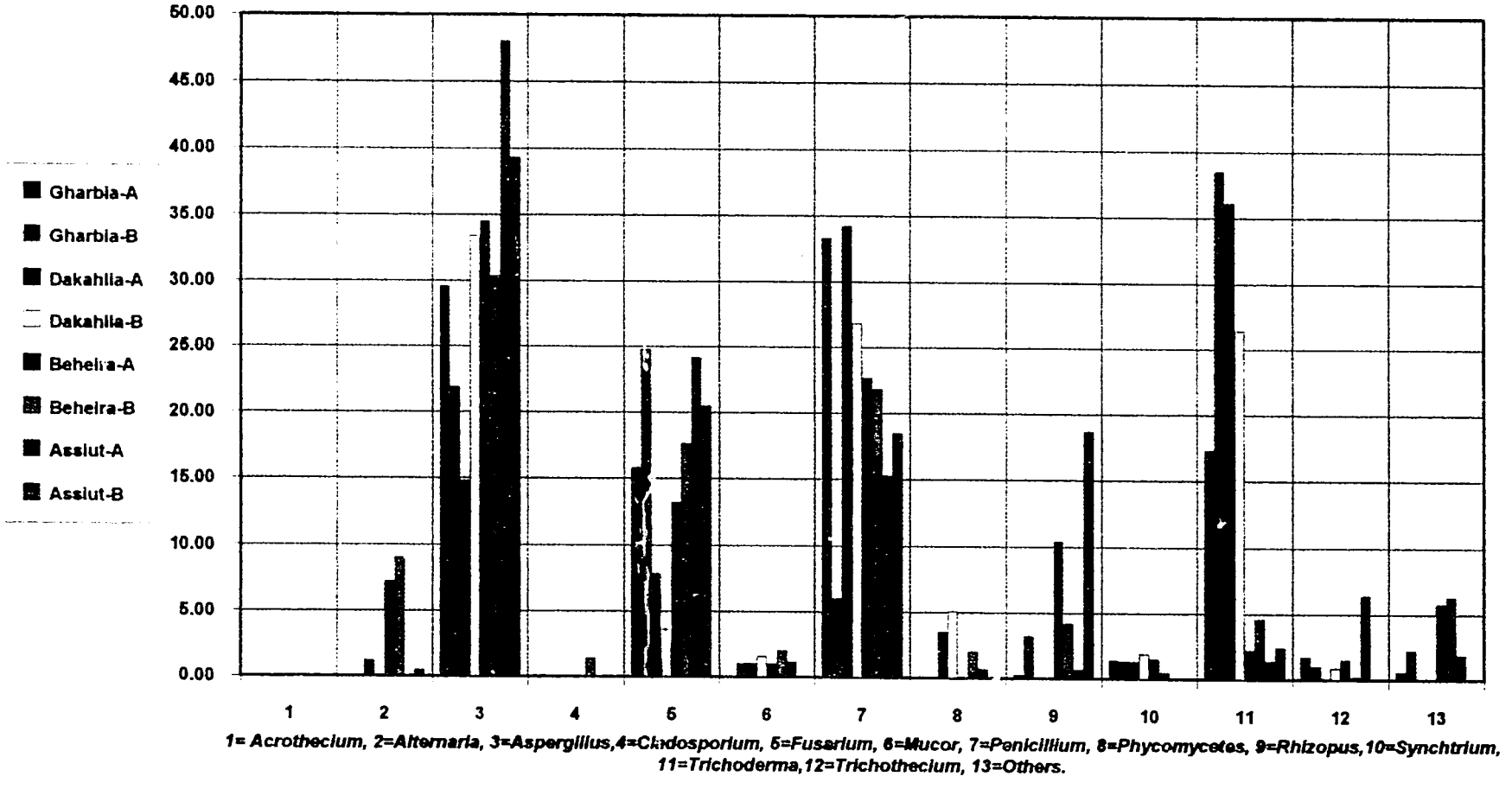


Figure 10. Frequency of occurrence of fungi in the rhizosphere of [A] plants infected with *Orobanche* sp. and [B] healthy plants in different governorates in Egypt during the period extending from April to June 1994.

disease. We tried to isolate from the youngest possible infected *Orobanche* shoots or tubercles, in the hope of isolating the largest proportion of primary pathogens.

b. Isolation techniques. Small pieces (*ca* 3 x 3 mm) of infected tissue were excised and surface sterilized in 1% sodium hypochlorite for 4 min, then washed three times in sterile glass-distilled water on either Martin's agar or (PDA) containing 50 mg/L chloramphenicol (to suppress bacterial growth). After 3 d (PDA) or 6 d (Martin's medium) incubation at 25°C, each appearing fungal colony was transferred to a fresh plate of PDA. Later single spore isolates were made to ascertain purity of the isolates.

c. Types of fungi appearing. A wide array of fungi appeared in the isolates from infected *Orobanche* (by microscopic examination) (Table 17). Every infected plant had at least one colony of *Fusarium*, many had *Alternaria* and of course many were not easily identifiable. We decided not to expend time or energy on taxonomic identification at this time. Identification will be done only on colonies that are either strong primary pathogens or strong facilitating secondary pathogens.

2. Pathogenicity testing.

a. Culture of plants. During this year we have used two common hosts of *O. aegyptiaca*; tomato (*Lycopersicon esculentum*) cv. M 82 and lentils (*Lens culinaris*) cv. D35. Both varieties are highly susceptible. Plants were cultured in greenhouses in "speedlinks" to the two-to-three-true leaf stage, the growth medium (peat, etc.) was washed from the roots by soaking and shaking under water. Two or three plants were placed on a 12 x 28 cm sheet of Whatman GFA glass fiber paper in a partially slit polyethylene file envelope with 40 ml of 0.5 Hoagland's nutrient solution, as in Activity II, Table 13. The plants were hung in a black plastic shrouded frame.

b. Inoculation with broomrape. After 6 to 10 d growth at 25°C, 12 h daylength, the envelopes were opened and the roots seeded with *ca* 60 mg seeds per envelope of surface sterilized *Orobanche* seeds (1% sodium hypochlorite + 0.01% Tween 20 for 10 min and three washes in sterile distilled water).

c. Inoculation with fungi. Twenty days later, after there were visible tubercles on the roots, plants were inoculated. The mycelium from one or two petri plates was scraped with a scalpel into 5 ml of glass distilled water containing 0.02% Tween 20 (to facilitate dispersion) and ground 30 s in a Heidolph Ulta-turrax homogenizer. The dispersion was sprayed on the roots of five envelopes. Each culture envelope was sprayed three times, when the *Orobanche* formed tubercles, and 6 and 12 d later. Fungal growth on the roots and tubercles was followed.

Table 17. Isolation of fungi from diseased *Orobanche*.

Isolate Accession Code	Type	Host	*Orobanche*	Location
EM_1*,EP_4,EP_6,EP_{16}	*Alternaria*	chickpea	*aegyptiaca*	Neve Yaar
EM_2*,EM_3,EM_{17},EM_{19},EM_{20}				
EM_{21}*,EP_{23},EP_{24}*,EM_{26},EM_{28}				
EP_2*,EP_5*,EP_{11},EP_{12}	*Fusarium*			
EM_4*,EM_{18}*,EP_1*,EP_3	unknown			
EC_4	*Alternaria*	melon	*aegyptiaca*	Shavei Zion
E_{1a},P_{2a},E_{3a},E_{1c}	*Fusarium*			
E_{4b},E_{4d}	unknown			
CM_1,CM_{21},CP_1	*Alternaria*	sunflowers	*cumana*	Afula
CM_3,CM_4,CM_{10},CM_1,CM_{14}	*Fusarium*			
CM_{15},CM_{16},CM_{17},CM_{18},CM_{19}				
CM_{20},CM_{22},CP_3,CP_{23},CP_{26},				
CP_{27},CP_{28},CP_{29}				
CM_{12},CM_{13},CM_{21},CM_{24}	unknown			
E_{6a}	*Alternaria*	tomato	*egyptiaca*	Balfouria
E_{5a},E_{5b},E_{6b},E_{6c}	*Fusarium*			
E_{6d}	unknown			
E_{7c},E_{8b},E_{9a},E_{10b},E_{11g},E_{12c}	*Alternaria*	peanuts	*egyptiaca*	Habonim
E_{7b},E_{7d},E_{8c},E_{9b},E_{10a},E_{10c},	*Fusarium*			
E_{10d},E_{11a},E_{11b},E_{11c},E_{11d},				
E_{11f},E_{11g},E_{12a},E_{12b}				
E_{7a},E_{8a},E_{8d},E_{10e},E_{10f}	unknown			
E_{11e},E_{12d},E_{12e}				

*Fungi that have already been checked for pathogenicity on *Orobanche*. None were found to be primary pathogens of broomrape (with tomato as host).

The P.I. of this project met with the Egyptian coordinator of the project in Amsterdam at the *Orobanche* meetings, and met with the Egyptian counterparts on this activity in Cairo. As both sides were just setting up their systems, it was decided that the best time for exchange would be at the annual meeting, when each side can assess how to further interface beyond the fruitful discussion we had.

3. Work plan.

During the next 6 mo, we plan to set up a sunflower system and further refine the growth system. We also plan to screen all the pathogens to ascertain which are primary, and place them in groups of low, medium, and high pathogenicity.

During the following 12 mo, we plan to screen those organisms that are not primary pathogens together with chosen, easy to grow, primary pathogens of each group to determine which are facilitating secondary pathogens, i.e. greatly improve the pathogenicity of the primary pathogens.

Throughout this period we will continue to collect putative pathogens from different crop/broomrape combinations throughout the year.

4. Constraints/Problems/Delays.

One delay was in getting started, due to the time span between the retroactive ratification of the project, and the receipt of the sub-contract in Israel. Another was that groups abroad that had promised to supply mycoherbicidal organisms for this research did not come through.

Activity IV. **Increasing Host Root Resistance to *Orobanche* Penetration by Elevated Expression of Caffeoyl-CoA-3-0-Methyl Transferase in Transgenic Crops (Israel) and Anatomical Analyses of Parasite/Host Root Relations (Egypt).**

Egypt - E. A. Hassan (Principal Investigator) with **R. Brammall**, Canada (Consulting Collaborator)

A. Anatomical Analyses of Parasite/Host Root Relations.

1. Procedures.

Roots of susceptible faba bean cv. 'Giza 2' and cv. 'Toto' from Canada, grown in sand, were sampled at the supposed maximum lignification (post seeding/mature tissues), fixed, and preserved in formol-calcium fixative. Immediate hand sections were examined under light microscope to show how lignins and phenolics, pectin, and cellulosic compounds react colorly to specific staining.

Root tissues were examined for presence or absence of phenolic or lignin-linked materials using the following procedures:

a. Autofluorescence of fresh and/or preserved root sections under UV, green, and blue illumination. Under UV illumination, lignin-like materials and phenolics fluorescence were a bright blue to whitish color. Under green illumination, all phenolic containing structures (i.e. xylem vessels, phloem fibers, the casparian band in the endodermis, and the hypodermal cell walls) fluorescence was a bright red color. Under blue light illumination, the xylem vessels appeared a faint reddish color. The phloem fibers appeared a faint blue white, the Casparian band was inconspicuous, but the browned or modified cell walls of the hypodermis appeared a bright whitish-yellow.

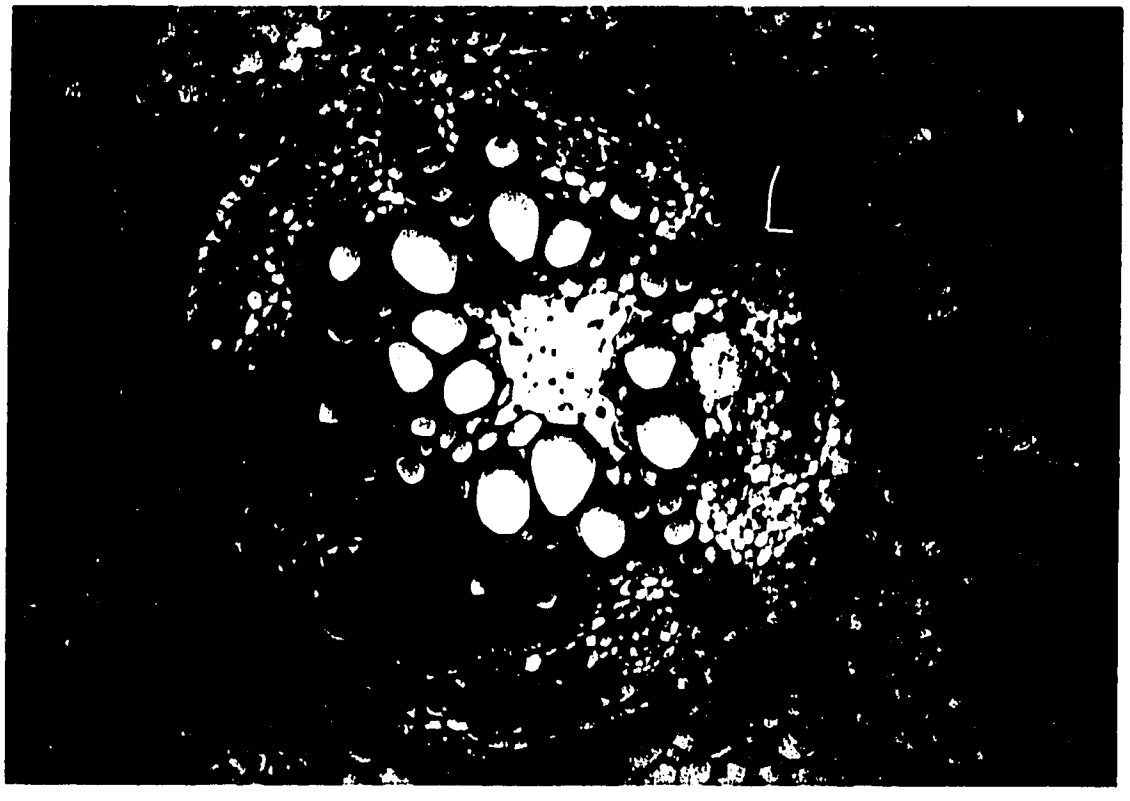
b. Mounting fresh or fixed, washed sections in phloroglucinol-HCl, a saturated solution of phloroglucinol (Sigma Cat. No. P3502) in 20% HCL-Lignin, appeared red-violet from the reaction of phloroglucinol with cinnamaldehyde residues.

c. The metachromatic reaction of cell wall components to toluidine Blue O (TBO Sigma Cat. No. T0394; 0.5% TBO (w/v) in 1% (w/v) sodium carbonate solution filtered using a 0.2 µm millipore filter immediately before staining. The fresh or fixed, washed sections were stained for approximately 30 s.

Lignin (i.e. those structures which are phloroglucinol-HCl positive) appeared blue-green to turquoise, and tissues containing other phenolics appear bright blue to dark purple. Pectin and cellulosic materials appeared pink to reddish (Figure 11A and B).

2. Results on the distribution of phenolic and lignin-like material in roots of susceptible faba bean Giza 2 (local/Egypt) and Toto (Canada) cultivars.

A



B

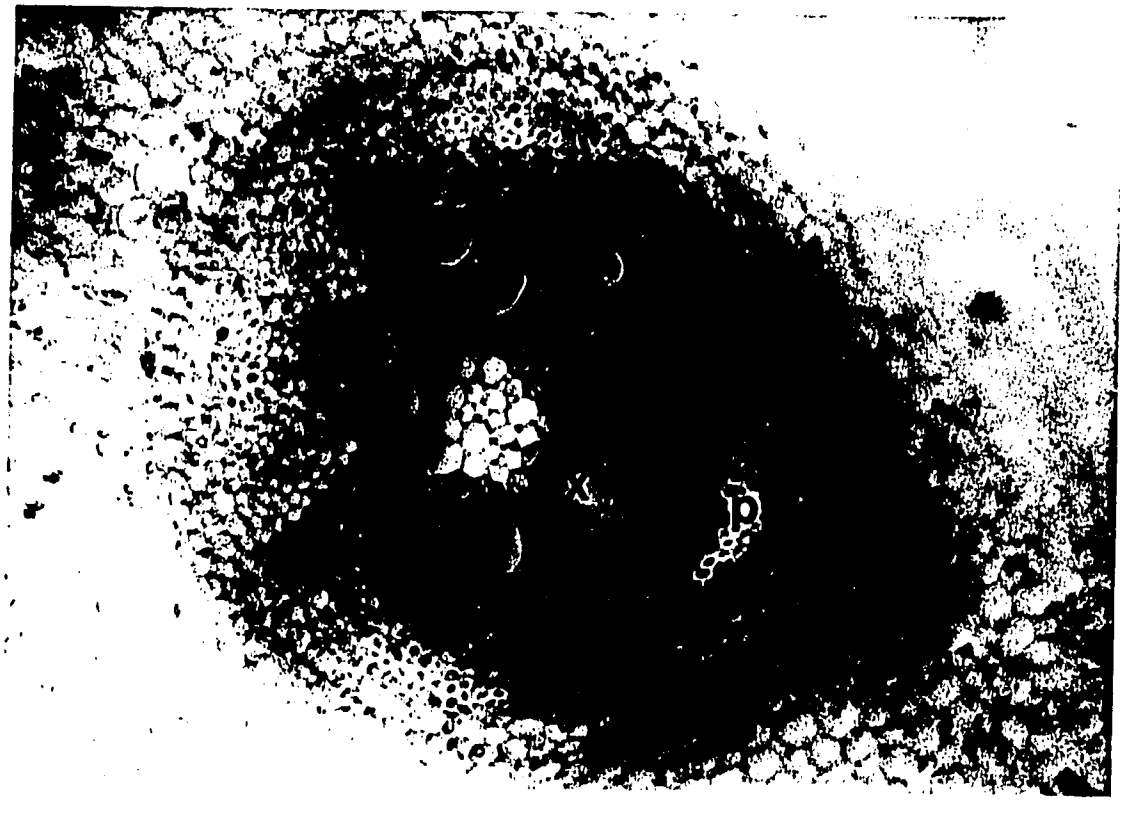


Figure 11. A. Visible light. Faba bean Giza 2 root section, stained with TBO. xylem = blue green, phloem = blue, cortical cell = pink. B. Phloroglucinol-CHL stained xylem vessels (tetrarch protostele).

The organization of the root tissues follows the typical dicotyledonous pattern. In the cv. Giza 2, the stele was a tetrarch protostele, but cv. Toto possessed a pentarch protostele (Figure 11A).

The outermost layer of the root is an ephemeral epidermis, below which is found a hypodermis. The outer tangential and radial cell walls of the hypodermis are a brown to reddish-brown color, suggesting the presence of phenolic components within them.

The epidermis and hypodermis are autofluorescent under ultraviolet, green, and blue light illumination indicating the likely presence of phenolic substances or suberin (Figure 12A and B and Figure 13A). The presence of phenolic compounds is further indicated by the dark purplish coloration of the hypodermal cell walls following staining with toluidine blue O. Staining with phloroglucinol-HCl is considered to be indicative of the cinnamaldehyde residues present in lignin (Figure 12 A and B). Phenolic or lignin-like materials were also detected using autofluorescent and phloroglucinol-HCl and toluidine blue O staining in the stele. Meta- and protoxylem vessels were highly autofluorescent and prominently stained with phloroglucinol-HCl. Thick-walled phloem fibers did not stain well with phloroglucinol-HCl but sometimes were a pink blue. They did show a bright blue (Figure 11 A) coloration with touidine blue O and were autofluorescent, indicating the presence of phenolics within their walls.

3. Conclusions - Implications for *Orobanche* infection processes.

Faba beans possess an autofluorescent hypodermis, (Figure 12) which possesses phenolic (or lignin-like) and lipidic (i.e. suberin-like) components. Both of these components usually make cell walls mechanically strong and resistant to cell wall degrading enzymes. Modified cell walls possessing suberin and/or phenolic components are often produced in tissues that are wound healing or responding to fungal infection which could be simulative to *Orobanche* penetration.

During penetration of the *Orobanche* haustorium, the parasite will initially have to pass through this layer before it can successfully establish connection to the host stele. There have been some suggestions that *Orobanche* seems to enzymatically dissolve away the host cell walls.

Following penetration of the haustorium through the hypodermis, the parasite must traverse the unspecialized parenchymatous cortex of the root. If the cortex is capable of responding to damage by modifying the walls of cells adjacent to the damaged site, through the deposition of phenolic and lipidic materials into them, then penetration is ceased. Successful infection would likely be determined by the rate at which the host cell wall could be modified and the speed with which *Orobanche* could pass through them. If the host responded very rapidly and sufficiently slowed the growth of the haustorium, the parasite might starve before it could enter the stele.

A

B

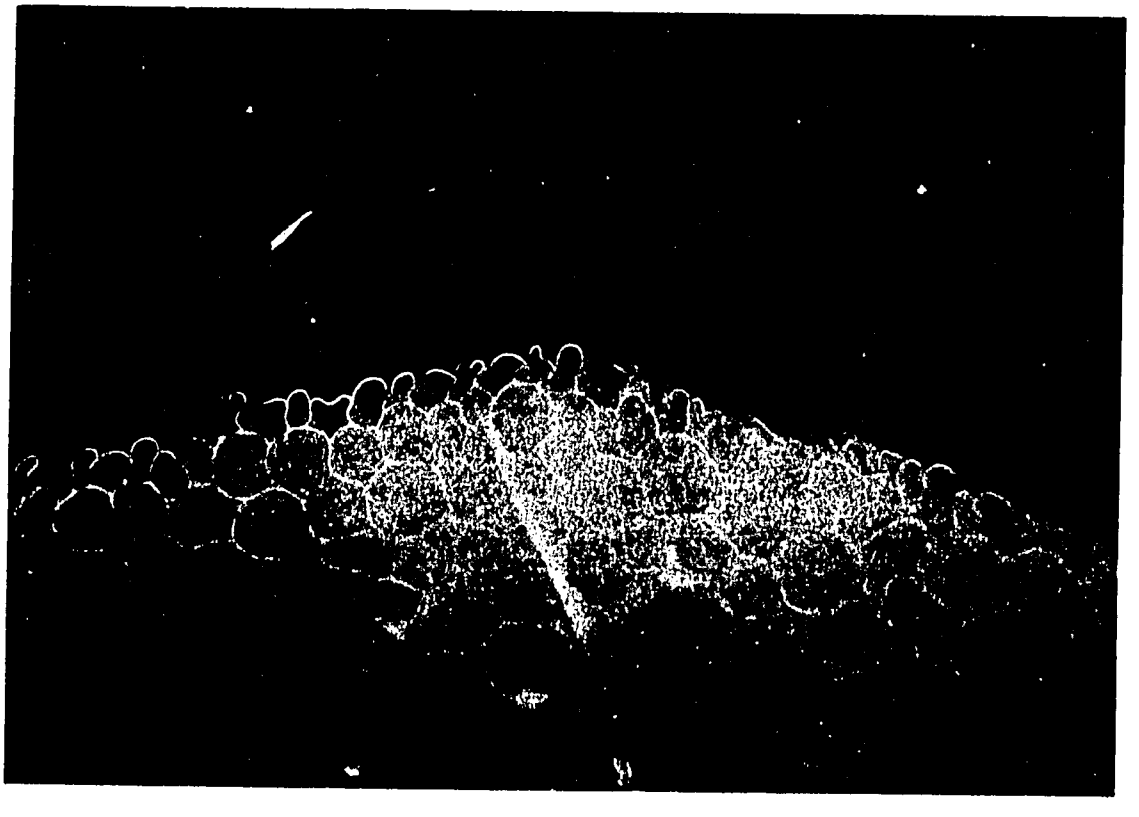
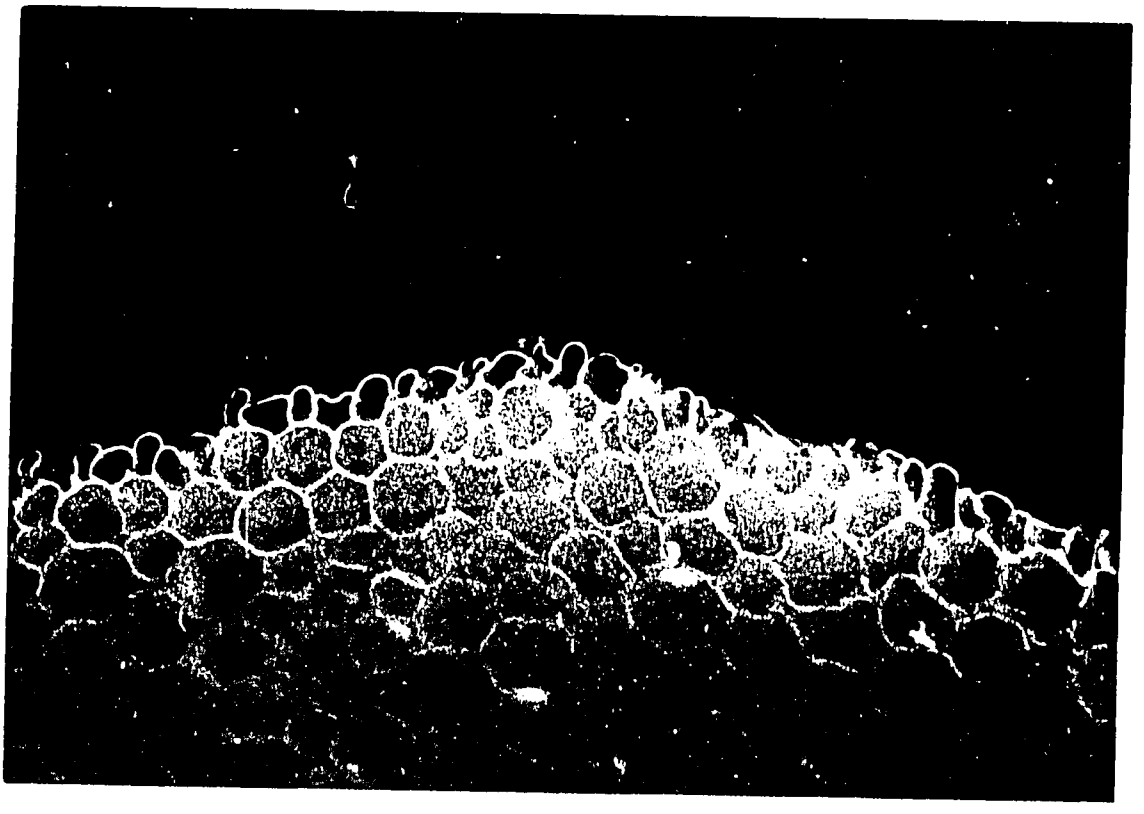


Figure 12. A. Under green light. Autofluorescent of faba bean outer root layers showing epiderms and hypoderms. B. Under blue light.

A



B

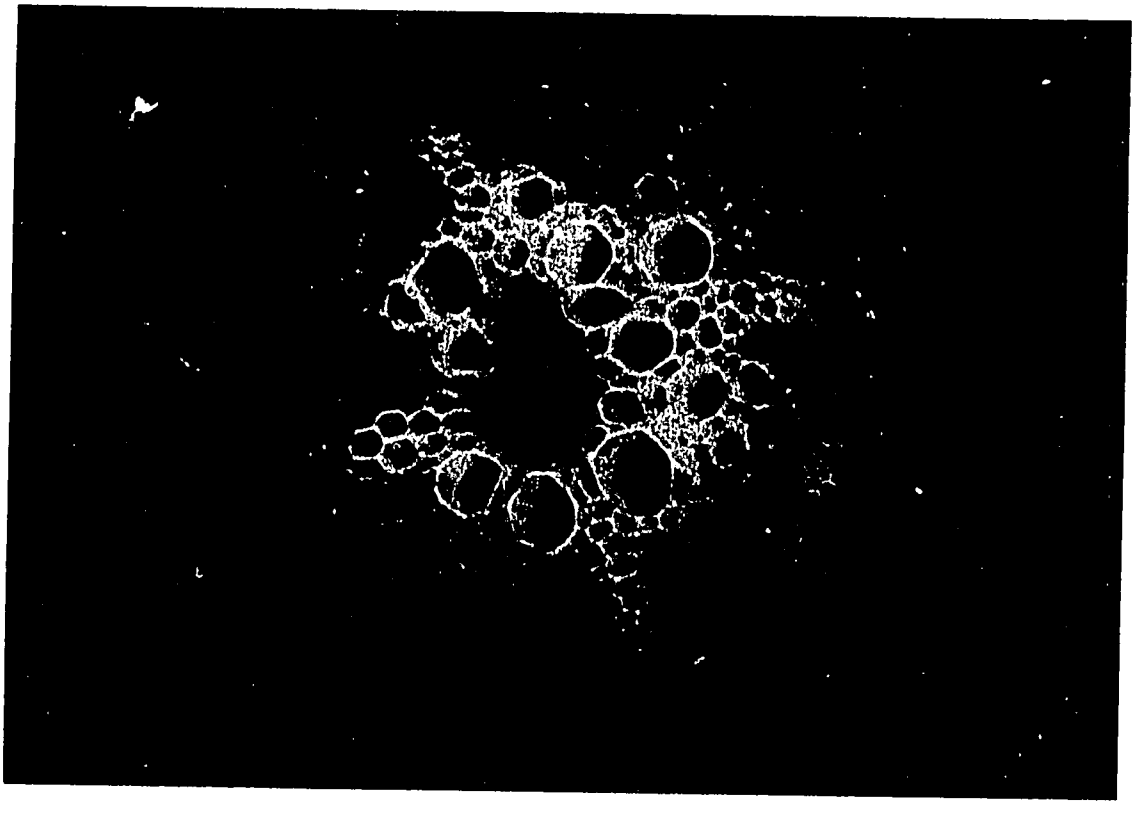


Figure 13. A. Autofluorescent of faba bean outer root tissues under UV light. B. Blue light fluorescence of stele: Xylem, phloem, and endoderms.

From results, faba bean hosts examined possess a well developed endodermis containing suberin and phenolic and lignin-like components. The regulatory role of the suberin in the passage of the solutes into the stele is well known. The parasite traverses this cell layer before the haustorial cells can make a connection with the xylem or receive nourishment from the host.

In case of host susceptibility (cultivars under test) *Orobanche* had the ability to overcome these natural defensive structural barriers within the host root. It is presumed that attempts to increase the ability of host cells to modify their cell walls with lignin-like materials during the penetration process would make the host more resistant to the parasite. There may be a race between the host and parasite; the host attempting to keep the haustorium from making a connection with the xylem and the parasite trying to establish this connection before the small food storage reserves in the *Orobanche* seed run out and it starves to death.

It should be noted that in our case because host and parasite are both angiospermous plants, it is very difficult for the host to detect biochemical triggers of the parasite because they are so similar. In the case of fungal infections, it often seems that the host is triggered to defend itself from infection when it "senses" the presence of fungal cell wall biochemical elicitors.

4. Plans for the next season, October 1994-1995.

   a. Installation and training on microscope operation system.

   b. Histochemical examination of healthy roots from different host plant species.

   c. Histochemical examination of samples from different infected host root species to detect and compare the changes in response to penetration and development of *Orobanche* and the localization of defense material in the root tissues.

Israel - A. Shomer-Ilan and **A. Zilberstein** (Co-principal Investigators)

B. Increasing Host Resistance to *Orobanche* Penetration by Elevated Expression of Caffeoyl-CoA-Methyl Transferase in Transgenic Crops.

The aim of this part of the project is to render crop plants tolerant to *Orobanche* attack by avoiding penetration of the parasitic haustoria into the roots of the host. Our approach is mainly based on the introduction of genes encoding enzymes which catalyze various steps in the phenylpropanoid pathway into crop plants to induce lignification or cell wall reinforcement. Our work has been focused on the introduction of the cDNA encoding caffeoyl-CoA-3-O-methyl transferase linked to either a constitutive or to a root specific promoter into tobacco. Tobacco is susceptible to *Orobanche* and therefore can serve as a model system for obtaining a fast estimation whether this

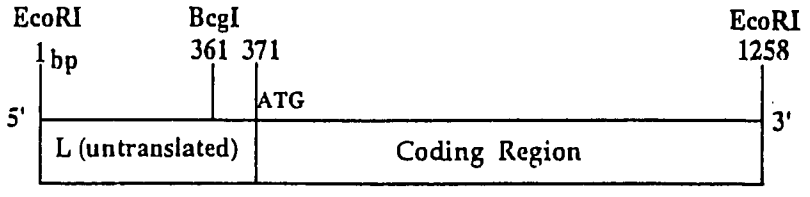
enzyme can be over-expressed in plants and its activity may exert relative tolerance to the parasitic attack by blocking the penetration of haustoria. The enzyme, caffeoyl-CoA-3-O-methyl transferase (CCoAMT), catalyzes the formation of ferulic esters from 4-coumaroyl-CoA, which is a central precursor in the phenylpropanoid pathway (Pakusch, et al. 1991). In certain plants the production of the enzyme is induced by pathogens or elicitors, resulting in cell wall reinforcement, which occurs gradually and requires several days. It is hypothesized that transgenic plants that constitutively express CCoAMT might have more resistant cell walls. Conversely, CCoAMT over-expression in all plant tissues might interrupt normal physiological processes and therefore its expression should be limited to the roots which are the target organs for *Orobanche* attack.

1. Construction of plant-Agrobacterium shuttle vector carrying the cDNA of CCoAMT linked to a constitutive promoter and tobacco transformation.

CCoAMT cDNA was isolated and characterized by Matern's group (Schmitt et al., 1991). This clone was isolated from a cDNA library obtained from mRNA isolated from elicitor- induced cells of parsley. The coding region of the CCoAMT was isolated from the pUC vector kindly provided by Prof. U. Matern (an advisor to this project), by cutting with EcoRI. This EcoRI fragment contained the coding region and 370bp 5' flanking region. This fragment was inserted in the EcoRI site of pGEM-7Zf (Promega) (Figure 14). The coding region was then isolated from pGEM-7Zf as a BcgI -SmaI fragment. BcgI cuts the 5' flanking region 10bp upstream to the translation start site and the 3' protruding end was then flushed by the exonuclease activity of T4 DNA polymerase. The coding region was cloned in BalI site of pRT103 between the CaMV 35S promoter and terminator sequences (Figure 15). This step formed the linkage between the CCoAMT coding region and plant transcriptional control signals which leads to constitutive transcription in most plant tissues. This cloning in pRT103 also enables further cloning of the CCoAMT coding region downstream to more specific promoters (see below). The Hind III fragment from pRTAMT carrying the coding region of CCoAMT and the 35S promoter and terminator, was cloned in the Hind III site of the *Agrobacterium*-plant shuttle vector pGIF2-Hind III. This vector, recently constructed in our laboratory, carries the PAT gene conferring resistance to the nonselective herbicide glufosinate.

The final construct pGIF-AMT(-L) (Figure 16) was transformed to the *Agrobacterium* strain GV3101-pMP90RK (Koncz and Schell, 1986), by electroporation. The *Agrobacterium* transformants were then used to transform *Nicotiana tabacum*. At present, the transformed leaf discs develop transgenic buds.

CCoAMT cDNA :



Insertion into EcoRI site of pGEM-7Zf.

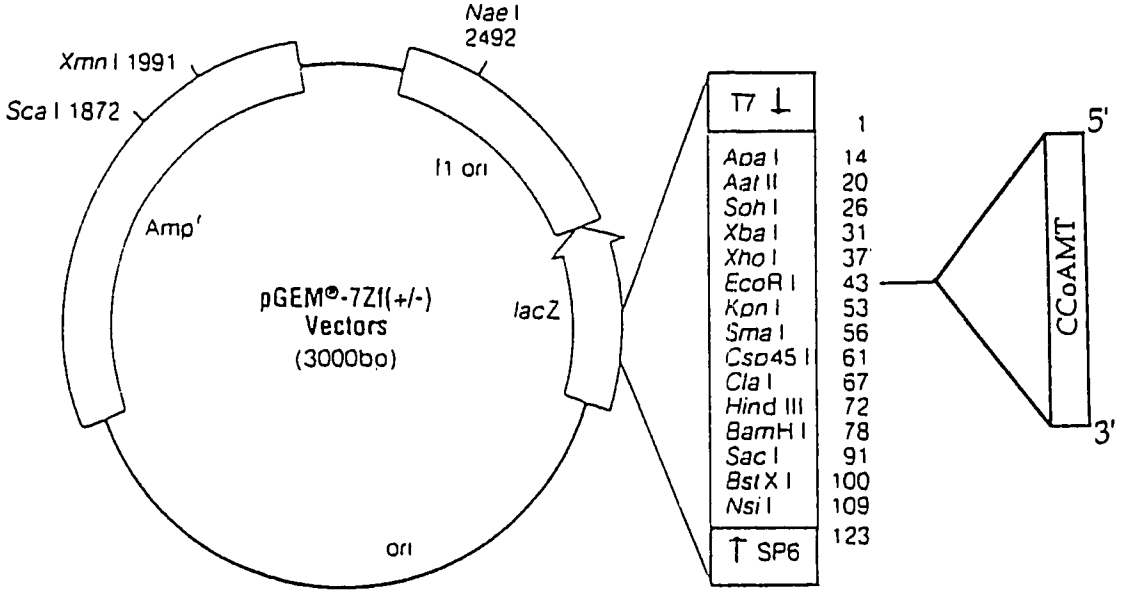


Figure 14. Cloning of CCoAMT cDNA fragment in pGEM-7Zf. The EcoR1 fragment comprising the coding region and the unstranslated leader is depicted in the upper panel. The lower panel describes the final construct.

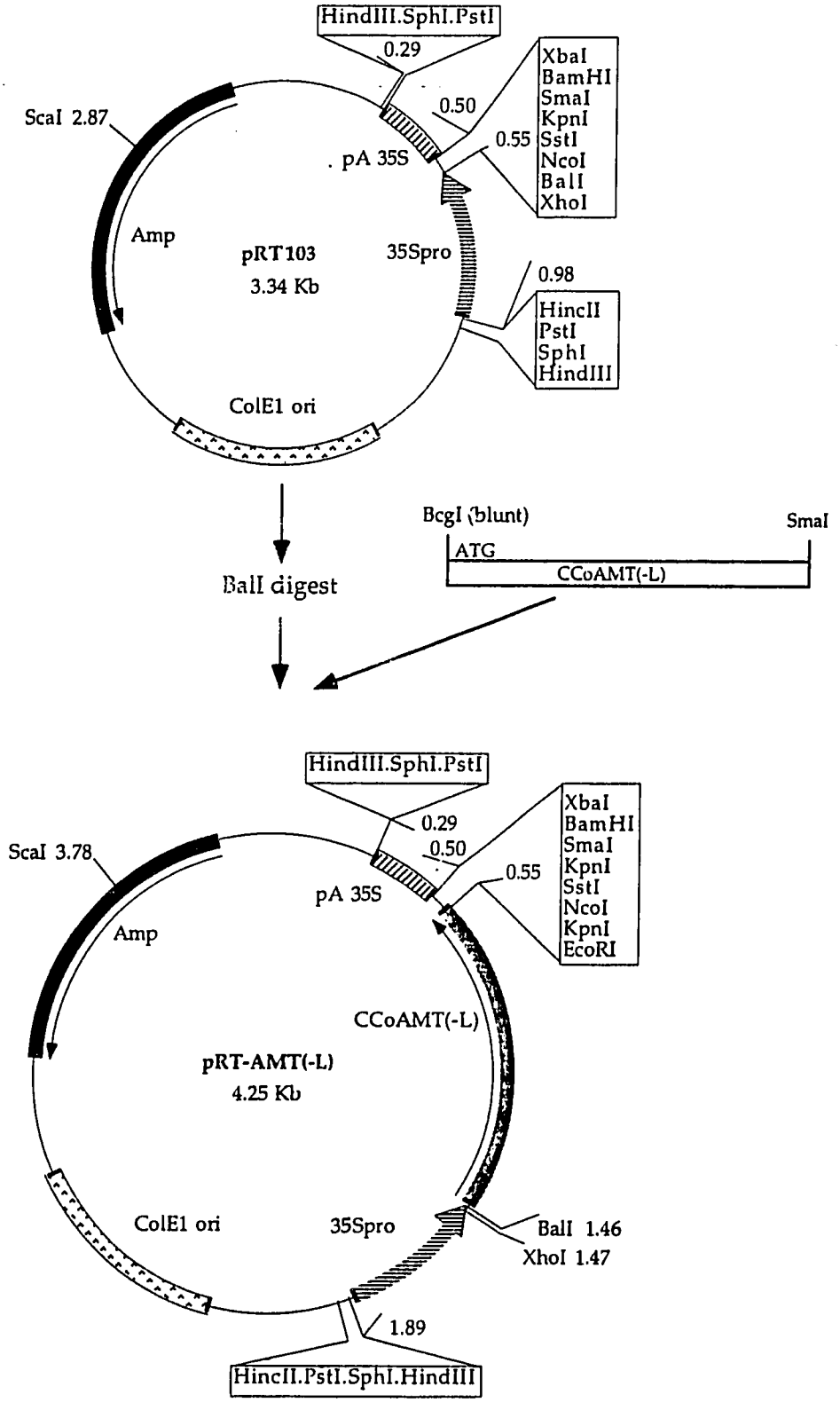
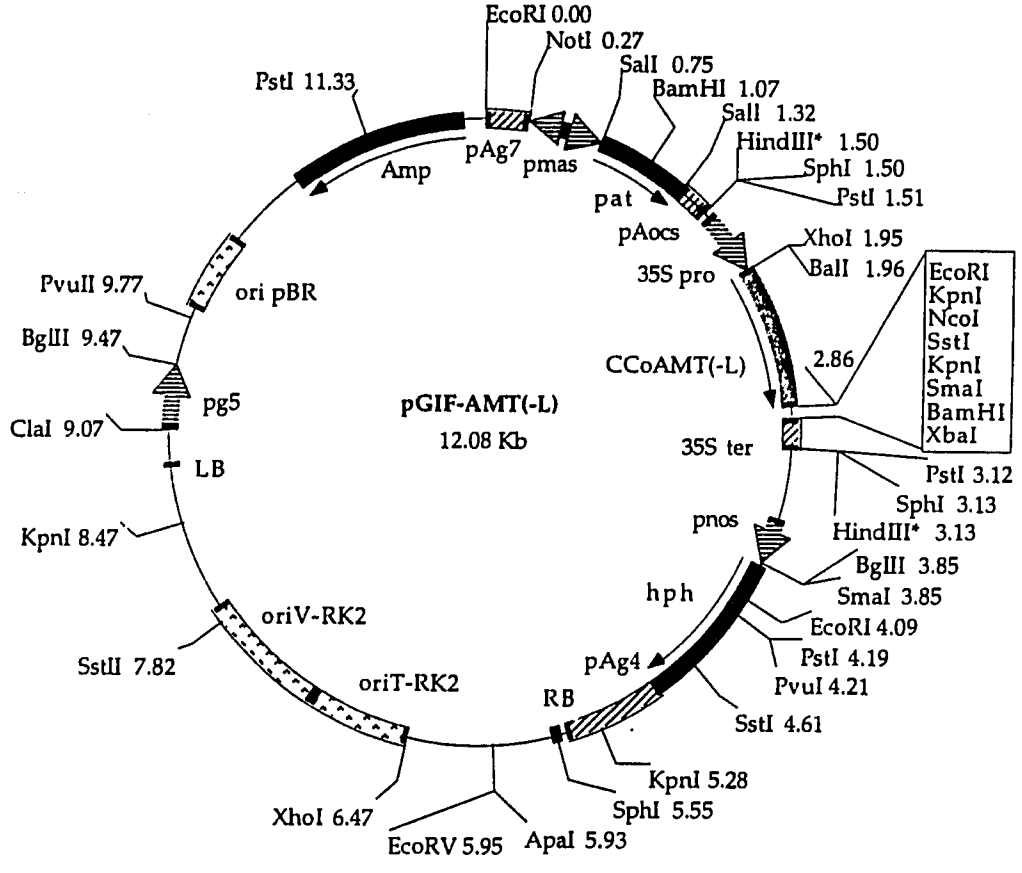


Figure 15. Insertion of the CCoAMT coding region between the 35S promoter and terminator in pRT103. The lower panel describes the resulting vector and the restriction sites flanking the CCoAMT coding region and the 35S sequences.



Plasmid name: pGIF-AMT(-L)
Plasmid size: 12.08 kb
Constructed by: Relly
Construction date: February, 1993

Figure 16. Detailed description of the *Agrobacterium*-plant shuttle vector carrying the coding region of CCoAMT. The vector belongs to the pPCV binary vector system (Koncz and Schell, 1986), harboring the hygromycin phosphotransferase gene as a selectable marker and the *pat* gene conferring glufosinate resistance in plants. The CCoAMT gene is situated between the two selectable markers.

2. Construction of a vector carrying the CCoAMT coding region linked to a specific root promoter to specifically express CCoAMT in root tissue.

A root specific promoter, containing the 35S minimal promoter fused to the glb3 enhancer (Szabados et al. 1990) will be used. Cloning experiments are currently carried out to construct an *Agrobacterium*-plant shuttle vector that will contain the CCoAMT driven by the root specific promoter.

3. Construction of cDNA library from roots of *Orobanche*-resistant sunflower.

During this year we have started constructing a root cDNA library from mRNA isolated from roots. In parallel, physiological experiments were carried out to characterize the time course of the defense response exerted by sunflower roots which are resistant to *Orobanche* attack.

During the second year of the research, a cDNA library will be constructed, using mRNA extracted from *Orobanche*-induced sunflower roots as a template. Further analysis of early responsive clones isolated from this library, will lead to the understanding of early events induced by the parasitic haustoria and to linking the genes encoding enzymes of the phenylpropanoid pathway to more specific promoters.

4. Collaboration.

Histochemical studies of root sections originated from diverse population of *Vicia faba* plants revealing differential resistance to *Orobanche*, have been performed by Prof. E. Hassan (NRC, Dokki, Cairo). In these studies, a correlation between lignification of cell walls and tolerance to *Orobanche* was observed. This anatomical research has achieved two goals that are complementary to the molecular approaches: a) confirming the linkage between cell wall lignification and resistance to *Orobanche* and b) acquiring histochemical techniques that will be used to analyze transgenic plants over-expressing CCoAMT. This analysis will help localize changes in cell wall composition in different root tissues.

5. Work plan for the next 6 and 18 months.

a. Development of transgenic tobacco expressing the coding region of CCoAMT driven by the 35S promoter (next 6 mo).

b. Completion of constructing an *Agrobacterium*-plant shuttle vector with the root specific promoter linked to the coding region of CCoAMT (next 6 mo).

c. Development of transgenic plants expressing the alien CCoAMT in root tissues (next 18 mo).

d. Analyzing transgenic plants for their cell wall reinforcement and resistance to *Orobanche* invasion (next 18 mo).

e. Construction of *Orobanche*-induced cDNA library from sunflower roots that are resistant to the parasite. Characterization of early induced genes (next 18 mo).

6. Requested changes in the budget for the second year.

The total budget approved for two Israeli P.I.'s groups (20,250 each) is far less than required to carry out the planned work because of the following reasons: Molecular biology studies are relatively expensive; enzymes, precursors, radioactive precursors, disposables, cassettes, films, chemicals, and many other required materials are imported from U.S.A. and Europe and the prices are 1.3-to-1.5-fold higher than in the U.S.A. Oligonucleotide primers that are essential for the work are charged $5 per nucleotide. The expenses required to cover the cost of greenhouse space supplies by the Botany Dept. for the transgenic plants, as well as other local services and equipment maintenance, are higher than previously estimated. The salary of the advanced post-doctoral fellow with experience in molecular biology, transformations, tissue culture and biochemistry is about $36,000 per year. In addition, two M.Sc. students are required ($13,000 per each). Until now, part of the salaries were paid by the University to support the commencement of the research but most of it should be paid by the grant budget during the second year. The budget has been reduced twice during preparation and therefore we would greatly appreciate a 25% increase in the total sum allocated to the molecular biology project of this Activity IV.

References

Koncz, C. and J. Schell. 1996. Mol. Gen. Genet. 204:383-396.

Pakusch, A. E., U. Matern, and E. Schiltz. 1991. Plant Physiol. 95:135-143.

Schmitt, D., A. E. Pakusch, U. Matern. 1991. J. Biol. Chem. 266:17416-17423.

Szabados, L., P. Ratet, B. Grunenberg, and F. J. deBruijn. 1990. Plant Cell 2:973-986.

Activity V. **Generation of Transgenic Crops with Target-Site Herbicide Resistances to Control Parasitic Weeds.**

Egypt - S. A. Kahil, H. A. Saber, and **M. H. El-Sherbeeny** (Principal Investigators)

A. Faba Bean Genotype Variable Responses to High and Low Glyphosate Doses in Respect to *Orobanche* Stress.

1. Experimental Procedures.

Two sites were selected on naturally infested soil in Fayoum to test for glyphosate tolerance to *Orobanche* of different genotypes. If we could get a percentage of resistance to be concentrated in one genotype, then we can gain a higher yield in naturally infested locations via resistance to glyphosate for control plus tolerance to the broomrape.

Nine faba bean lines varied in their tolerance to *Orobanche* when exposed to high and low doses of glyphosate as follows:

Two on-farm researcher-managed trials were planted in natural infested soils with *Orobanche* at Fayoum governorate to evaluate the performance of the faba bean breeding lines, (Giza 2, Giza 402, Giza 429, and Giza 674) under high and reduced rates of glyphosate and its effect on *Orobanche* control. Complete randomized blocks with four replicates were used. The recommended rates for faba bean were applied to both trials and the following treatments were tested:

1 - Check control	
2 - Giza 2 + glyphosate (649 a.i./ha)	twice
3 - Giza 2 + glyphosate (349 a.i./ha) + NPK	twice
4 - Giza 402 + glyphosate (649 a.i./ha)	twice
5 - Giza 402 + glyphosate (349 a.i./ha) NPK	twice
6 - Giza 429 + glyphosate (649 a.i./ha)	twice
7 - Giza 429 + glyphosate (349 a.i./ha) + NPK	twice
8 - Giza 674 + glyphosate (649 a.i./ha)	twice
9 - Giza 674 + glyphosate (349 a.i./ha) + NPK	twice

2. Results.

Varieties differed in their response to glyphosate (Table 18). The varieties susceptible to *Orobanche*, Giza 2 and Giza 402, showed lower tolerance to glyphosate. The *Orobanche*-resistant ones, i.e. 429 and 674 showed higher tolerance to glyphosate as indicated by seed yield. Confirmation next season is needed and includes more than 200 lines in a breeding program and different doses of glyphosate.

Table 18. Tolerance of faba bean genotypes to glyphosate under *Orobanche* infestation.

Faba bean genotype/ treatment	*Orobanche* Weight (t/ha)			*Orobanche* Number			Host Seed yield (t/ha)		
	Site 1	Site 2	Mean	Site 1	Site 2	Mean	Site 1	Site 2	Mean
1	2.117	1.705	1.911	723.333	793.571	758.452	0.476	0.343	0.410
2	0.352	0.629	0.490	145.476	290.476	217.976	1.726	1.788	1.757
3	0.662	0.805	0.733	294.762	375.000	348.881	1.660	1.633	1.662
4	0.388	0.490	0.439	184.524	239.762	212.143	1.660	1.943	1.801
5	0.574	0.771	0.673	160.714	419.047	289.881	2.155	1.824	1.989
6	0.348	0.293	0.320	145.746	147.619	146.547	2.726	2.114	2.420
7	0.436	0.495	0.465	278.571	259.762	269.166	2.750	2.771	2.761
8	0.460	0.474	0.467	236.905	395.238	316.071	2.121	2.050	2.086
9	0.727	0.571	0.472	211.905	434.523	323.214	2.145	1.845	1.995
C.V. %	26.60	41.37	35.29	96.11	38.37	65.675	17.15	21.40	19.256
L.S.D.	0.25	0.41	0.247	N.S.	208.36	224.8	0.48	0.56	0.381

3. Plans for next season (1994/1995).

a. At Sids Experimental Station, our aim is to conduct studies with Giza 429 and 674, which gave the highest yield and level of resistance to *Orobanche* to increase their tolerance to glyphosate.

b. Laboratory and greenhouse tests for glyphosate resistance in other hosts, (exposure to glyphosate stress) will be carried out.

c. We will attempt to obtain mutagenic seed material, through direct application of chemical mutagens in the field, to test for glyphosate resistance. Ethylmethane sulfonate (EMS) and other chemicals will be examined.

4. Procurement/Lab establishment.

A new laboratory (wall and floor) has been offered by the Centre on April 4, 1994. Benches and laboratory utilities, including water, electricity, and telecommunication were furnished through the project budget (services item). Designated equipment was purchased and delivered as follows:

Description of Item Equipment 63,379 $ = 42.95% of the total*

Item	Activity	Cost	Delivery
1. Camera	Act. I	1,376	✓
2. Refrigerator		577	✓
3. Computer		3,735	✓
4. Photocopier		1,704	✓
5. Electric typewriter		955	✓
6. Overhead projector		430	✓
7. HPLC/down payment Shipped from USA		15,000	-
8. Incubator	Act. III	2,611	-
9. Epifluorescent microscope Down payment	Act. IV	27,000	✓
Total		63,379	

*Total money received Sept. 1993 - Sept. 1994 = 147,538
Effective deposition April 1993.

5. Travel.

a. In November 1993, the three coordinating scientists from the U.S., Egypt, and Israel along with most of the Israeli activity P.I.s met at an International Broomrape workshop in Amsterdam, where we held a special coordination session as well as informal meetings.

b. From Israel to Egypt Jan. 1994.

Profs. Galun and Gressel went from Israel to Egypt for further project coordination and met with all the Egyptian scientists and administrators for fruitful meetings. Preliminary arrangements were also made for two of the Egyptian scientists to visit Israel during *Orobanche* season, but this unfortunately did not come about despite all arrangements being made.

c. From Egypt to Israel July 1994.

The project bridged to an official visit from the Centre in July 1994. (Please see enclosed next page). The visit was organized/coordinated by Prof. Gressel and Dr. Esmat Hassan and included her meeting with all members of the project in Israel.

The inclusion of new research institutions in addition to Weizmann Institute in the program of scientific cooperation between Egypt and Israel, in the NRC (Egypt) is being considered. The Cooperation is now under discussion on the level of different Divisions and Departments. Because the Centre already has scientific cooperation programs with the other countries in the region, cooperation will be easier on a regional level in the future with Egypt, Israel, and any of the Arab countries.

6. Collaboration.

The first year of the project allowed good recognition of the personnel through short visits. Collaboration, which included working together and sharing publication of scientific results, needed a longer stay (no less than one month) on both sides. This is considered in next year's plan.

7. Personnel changes (new personnel).

Mr. Sabry M. N. Mostafa was hired as Administrative Assistant; one secretary was hired also. Through him the project is in proper and regular contact with the huge administrative system of the Centre. His job includes managing all purchase communications, filing, travel facilities, banking and budget documentation, bookkeeping, etc. (full agenda of the project).

Dr. Hamdi Aziz Saber, Sh. Khalil and M. El-Sherbeeny, Legume Department, ARC - Min. Agric. were connected to Activity V.

Israel - D. M. Joel and **D. Aviv** (Co-principal Investigators) with **J. Gressel** (Consulting Investigator)

Two main kinds of resistance to herbicides can be conferred on crop species using biotechnology: metabolic resistance, where the resistant plant degrades the herbicide to non-toxic products thus preventing its harmful effect, and target-site resistance, where the target enzyme affected by the herbicide is modified in a manner precluding binding thus suppressing herbicidal activity without changing the normal function of the enzyme. We assume that when root parasites like *Orobanche* are to be controlled, target-site resistances are more relevant because only they permit safe movement of the herbicide *via* the host plant toward the parasite connected to its roots. This research is split between Neve Yaar, where the studies with transgenic plants are performed, and the Weizmann, where transgenic plants will be generated using pre-existing genes, and Egypt, where mutagenesis is being used to generate targetside mutant crops.

A. Part 1 - D. M. Joel.

1. Experimental progress.

Four transgenic resistances have been tested as models to examine the above hypothesis: (a) rapeseed with target-site resistance to glyphosate, based on a modified enolphosphate-shikimate phosphate (EPSP) synthase gene; (b) tobacco with the SURB-Hra gene, a modified acetolactate synthase (ALS) gene, that confers target-site resistance to chlorsulfuron; (c) tomato with metabolic resistance to glufosinate ammonium, an inhibitor of glutamine synthase, based on the *bar* gene that was taken from *Streptomyces hygroscopicus*. This gene codes for an enzyme that degrades the herbicide, i.e. resistance is not at the target site; and (d) tobacco with the *sul* I gene that encodes a modified dihydropteroate synthase (DHPS), conferring target-site resistance to asulam. Seedlings of these transgenic plants were selected with the respective herbicides, the resulting younger plants were then transplanted into pots filled with soil infested with seeds of *Orobanche aegyptiaca* (*ca* 5000 seeds per pot). During the development of the plants they were treated with various doses of the respective herbicides. Their development was followed and broomrape infestation was documented. As controls, we used treated non-transgenic plants as well as untreated transgenic plants, with and without broomrape.

As expected, the application of glufosinate-ammonium to transgenic tomato plants with the metabolic resistance to this herbicide failed to control *O. aegyptiaca* that parasitized its roots. The treated plants, like the controls, were heavily infected with the parasite and the yield was drastically reduced. Conversely, all three target site resistances proved effective. A single application of glyphosate to glyphosate-resistant

rapeseed infected with *Orobanche* resulted in a complete prevention of broomrape emergence, with concomitant normal growth and flowering of the transgenic host (Figure 17). Non-treated control plants were heavily infected with the parasite, and did not develop any flowers. These infected stunted plants died soon after the emergence of the parasite.

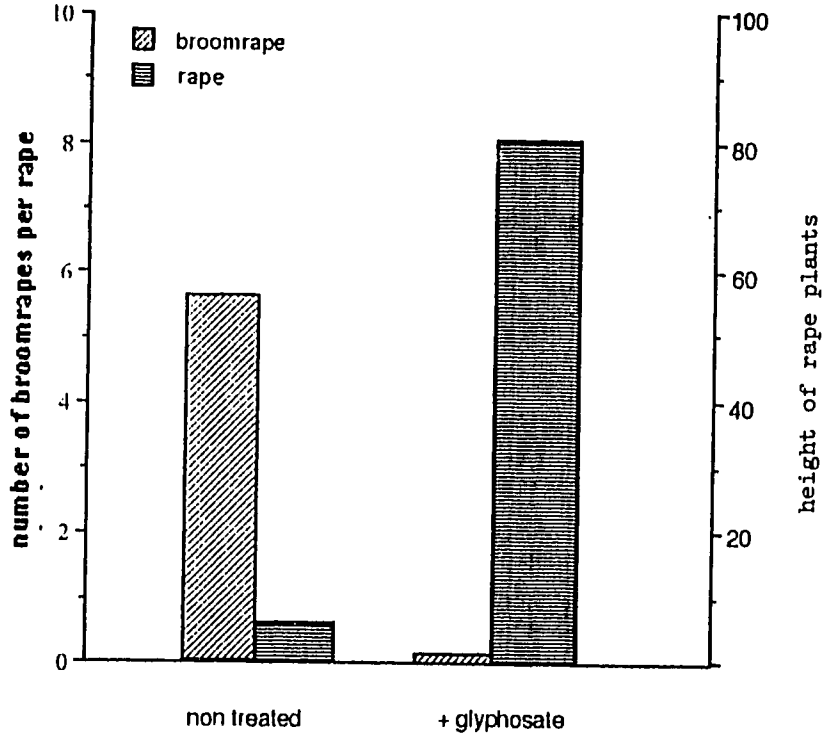


Figure 17. Influence of foliar application of glyphosate on height of rate plants infested with broomrape and on broomrape infestation of these plants.

In a similar manner broomrape was controlled on transgenic tobacco plants containing the SURB-Hra gene by foliar application of chlorsulfuron, as already reported by Joel (1992). All controls were heavily infected with the parasite, did not flower and reached not more than 50% of the normal height and shoot dry weight, while a single application of the herbicide resulted in normal growth and flowering of the transgenic host and the production of *ca* 95% of the normal tobacco yield.

With asulam resistance there is a considerable reduction in broomrape infection when high doses of the herbicide are applied to young tobacco plants (Figure 18). While 7 broomrape plants infected in average a non-treated control tobacco plant, foliar application of 8 kg ha^{-1} asulam, 26 days after planting, resulted in only 1.5 mature broomrapes per treated transgenic tobacco plant. Asulam application at later stages of tobacco growth were less effective.

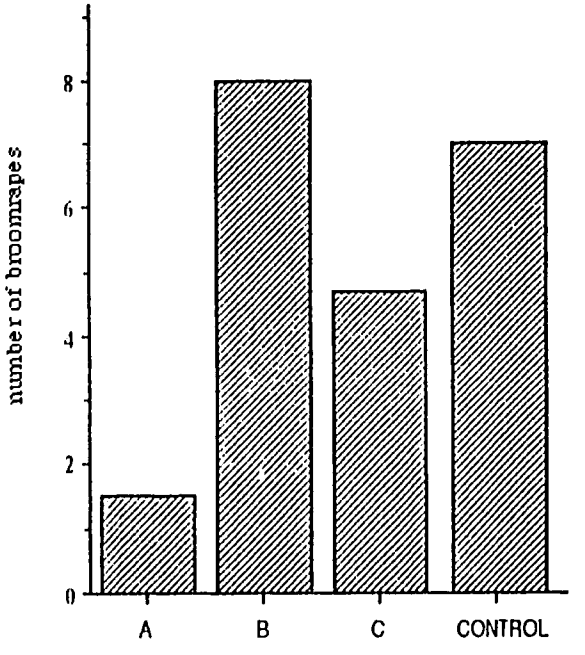


Figure 18. Average number of broomrape plants on asulam-resistant tobacco plants treated with 8 kg ha^{-1} asulam. A = 26d AP, B = 35d AP, C = 44d AP.

These results clearly demonstrate the potential of transgenic herbicide-resistant crops in the control of parasitic weeds as we have already reported preliminarily (Gressel et al. 1994). It is conceivable with these results that world agriculture could gain control over parasitic weeds if effort is put in transfer of the available target-site resistances into the genomes of local varieties of susceptible hosts of these parasites. Some herbicides, like glyphosate, are friendly to the environment, relatively cheap and were shown to be effective in relatively low doses.

The results of the first year contribute two main items to the project: data on relevant resistances that will be used in the planned transformations, and methodological knowledge of the optimal technical approach to testing herbicide resistances for broomrape control.

So far we have had one meeting with Dr. E. Hassan from Egypt, with whom we discussed our achievements, and the research planned in Egypt for pollen mutagenesis. A main issue was the methods for pollen collection and treatment. First trials with chemical pollen mutagenesis were already performed in Newe Ya'ar with sunflower. Seeds were collected and will be tested for ALS resistance during the next season. Another experiment is on its way at present. During the next two years we plan to extend these experiments for isolation of new ALS resistances.

2. Constraints.

A major problem that we faced during the first year is the delay in money transfer. The ARO does not provide any credit on the expense of promised budgets.
As overseas travel was not necessary during the first year, we have asked for a change in the use of the money, originally allocated for travel, to purchase a computer that is needed for accumulation of data, statistical analysis, communication with other institutions and preparation of reports and publications.

3. Plans for next 6-18 months.

We are waiting for permission to use gene constructs for transformation of plants as in the original research program. Once the first transformants will be available we will test them for broomrape control using the methods developed in Newe Ya'ar. Until then, we will refine the findings with the target site resistances in our hands for detailed publication, and perform preliminary field tests of their efficacy.

B. Part 2 - D. Aviv.

As stated in our proposal, we planned to obtain several herbicide resistant genes, from various sources and to use them for transforming different crops that are heavily attacked by the plant parasite broomrape. At an early stage, we received the first positive answer, from Rhone Poulenc, that the company was willing to give us the pJiT119 plasmid containing the asulam resistant gene. Unfortunately, mainly for bureaucratic reasons, we still do not have that plasmid in our hands; though we hope that soon we will have it. Recently, American Cyanamid in principle agreed to give us the mutant ALS gene, which imparts resistance to imidazolinone herbicides. So with these two resistant genes we will pursue our project.

So far, we refrained from hiring a senior scientist but with the understanding that shortly we will have at least one herbicide resistant gene, we hired Dr. Tamara Sorov, a new Russian immigrant, who joined the project on September 4, 1994.

Originally we planned for the first year transformation experiments using crop plants that are easy to transform, e.g. potato and tomato, and in parallel to develop methodologies for more recalcitrant crops e.g. *Vicia faba*. As mentioned above,

because of lack of herbicide resistant genes, we could not perform the transformation experiments. Therefore, I started with developing methodologies for recalcitrant crops and changing somewhat the proposal timetable; I started with sunflower. This work was done with the help of Mr. Ori Jacobson.

1. Transformation strategies for sunflower (*Helianthus annuus).*

a. Screening different varieties for regeneration ability. Twenty different sunflower varieties (kindly supplied by Mr. D. Itzhaki, a local sunflower breeder) were germinated aseptically and sections of their hypocotyls were incubated under the same conditions as specified by Paterson and Everett (Plant Science 42:125-132, 1985). All twenty lines produced calli but none regenerated plantlets. Under the same conditions we tested also a hybrid line (Florom-328), kindly provided by Dr. Menczel of Szeged, Hungary. This line was the only one to regenerate a few plantlets. Unfortunately, we have only a limited number of seeds of that line.

b. Screening local strains of *Agrobacterium tumfaciens* for "shooty" characteristics. Most wild type strains of *A. tumefaciens* induce undifferentiated crown galls on infected plants. A few "shooty" strains exist that cause their galls to develop shoots spontaneously. This phenomenon was used successfully by Juanin's group for poplar transformation thus developing a co-inoculation procedure (Brasiliero et al. Plant Mol. Biol. 17:441-452, 1991). The method was based on dual inoculation i.e. using an oncogenic strain that induced shoot differentiation and a disarmed strain that provided the engineered gene in a binary vector. To apply this procedure for sunflower we needed a "shooty" strain. Dr. D. Zutra, of the Volcani Center, kindly provided us with 18 different local isolates of *A. tumefaciens*. All induced calli, on infected hypocotyls, on hormone less medium. Unfortunately, none turned out to be of a "shooty" type.

c. Protoplast isolation. Because of previous experience in isolation and culture of protoplasts of several genera (e.g. tobacco, potato, tomato, citrus) and their use in the production of transformed plants, we recently attempted to isolate sunflower protoplasts. As starting material, we used one-day-old germinated seeds. We tested several enzyme combinations and different culture conditions. We succeeded in developing a procedure for the isolation of high yields of protoplasts. We also succeeded in inducing the first divisions (see attached Figure 19). Now we are attempting to further culture those small colonies into plant regenerating calli.

Figure 19. Ten-day-old sunflower protoplast derived colony.

2. Plans for next 6-18 months.

The addition of Dr. Tamara Sorov should contribute to a faster progress, in several directions: a) as soon as we can we will obtain the herbicide-resistant gene, we will transfer it into potato and tomato plants, b) we will continue to develop the sunflower protoplast system with the aim of obtaining plant regenerating calli, c) 6-18 mo we will start to develop also a protoplast system from *Vicia faba*.

3. Constraints.

We have been refused the gene construct for glyphosate resistance by Monsanto, and other U.S. companies with constructs for resistant genes have yet to come through. The direct contact of these companies by AID would be appreciated, as well as through indirect contact through ISAAA, an international organization funded by AID for the stated purpose of brokering between industry and developing countries for getting useful genes.

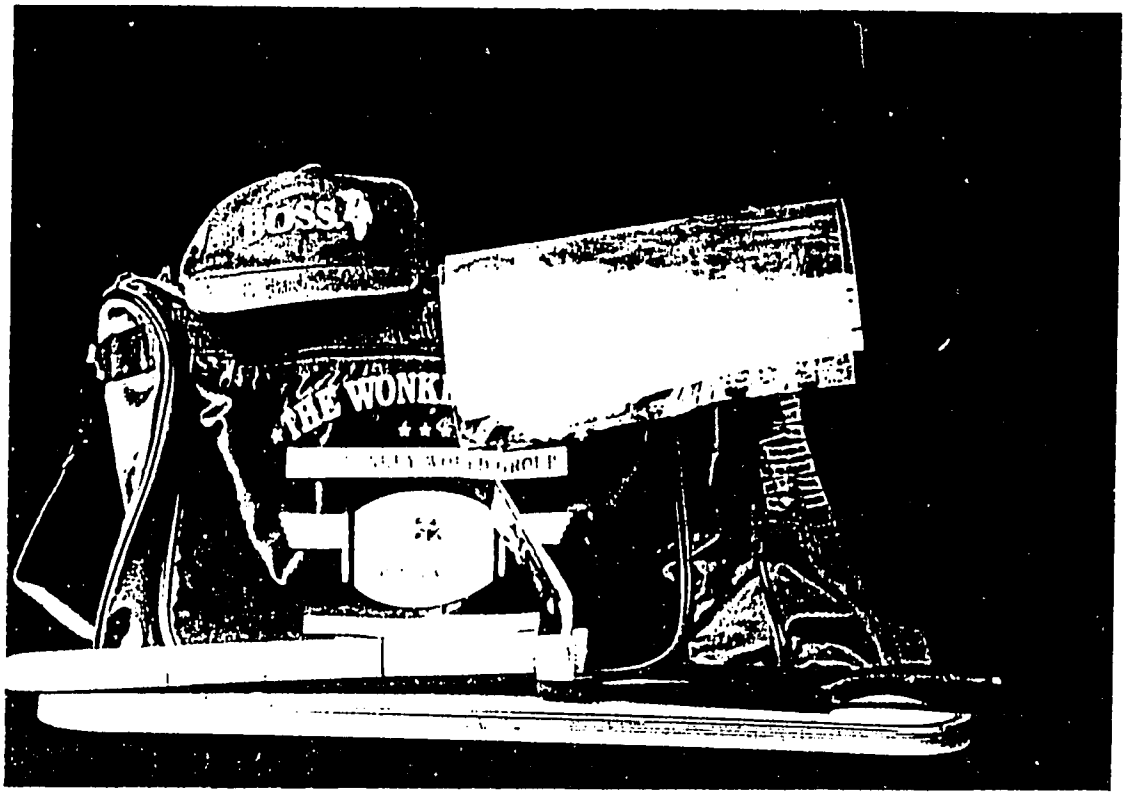
References

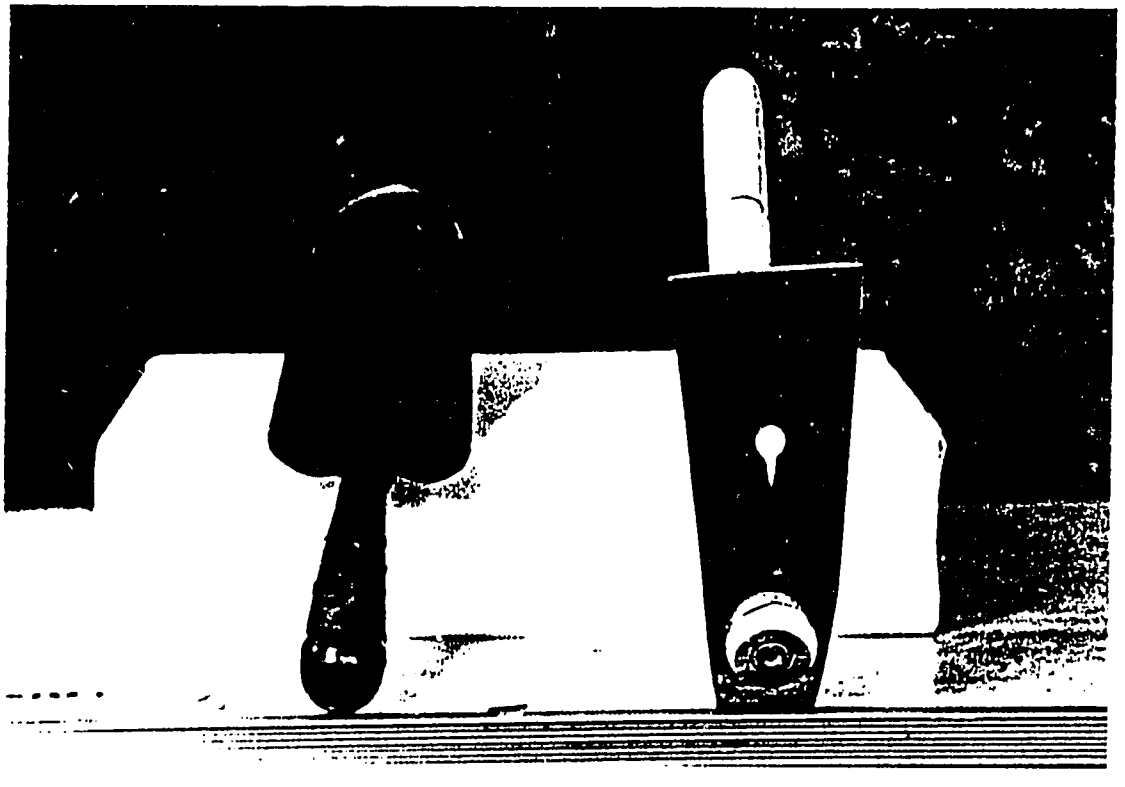
*Gressel, J., Y. Kleifeld, and D. M. Joel. 1994. Genetic engineering can help control parasitic weeds. In: Pieterse, A. H., J. A. C. Verkleij, and S. J. ter Borg (eds.). Biology and Management of *Orobanche* and Related *Striga* Research Proc. Third Intern. Workshop on *Orobanche*. Royal Tropical Inst., Amsterdam. (in press).

Joel, D. M. 1992. Control of broomrape *(Orobanche aegyptiaca* Pers.) with chlorsulfuron on a transgenic sulfonylurea-resistant crop. Weed Sci. Sco. Abst. 32:193.

*Citation emanating from this project.

Appendix





Activities I and III. Survey and sampling instruments.

Appendix 1

Activity I. Survey - Delta - El-Gharbia. *Orobanche* spp. on cabbage.

Appendix 2

Activity I. Survey - Delta - El-Gharbia. *Orobanche* spp. on tomato.

Appendix 3

Activity I. Survey - Delta - El-Gharbia. *Orobanche* spp. on clover.

Appendix 4

Activity I. Survey - Middle Egypt - Banysweif. *Orobanche* spp. on chamomile.

Appendix 5

Activity I. Survey - Middle Egypt - Banysweif. *Orobanche* spp. on geranium.

Appendix 6

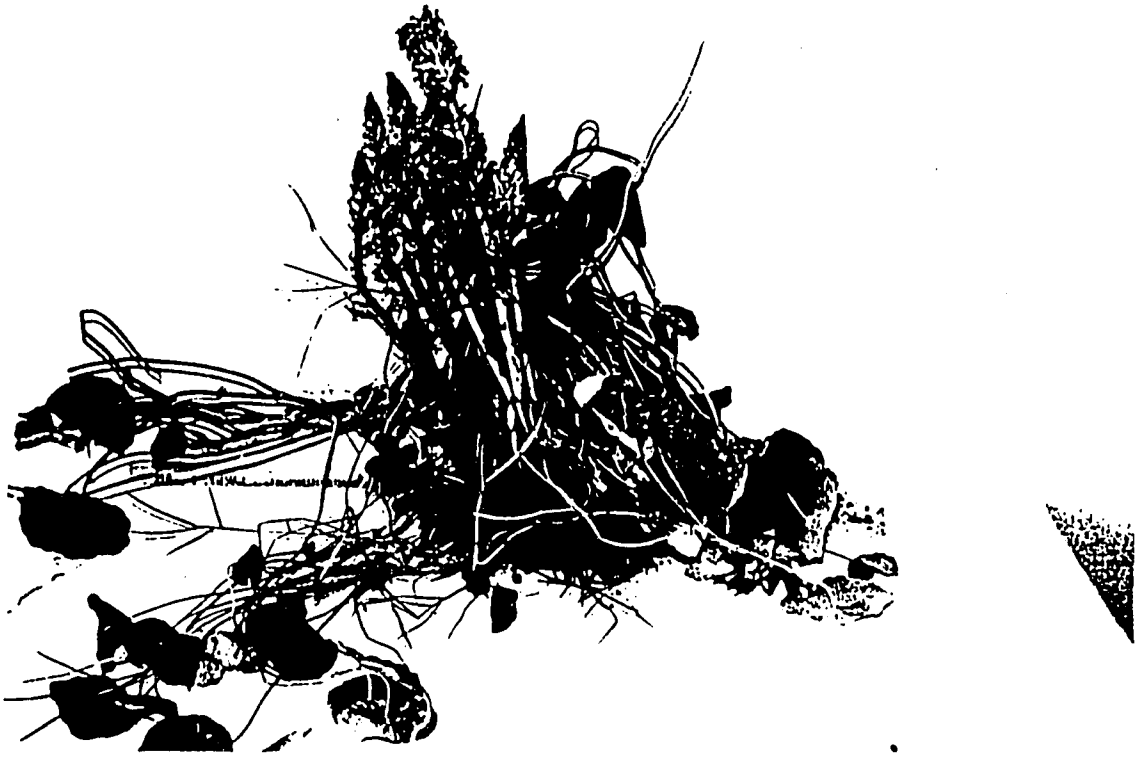
Activity I. Survey - Middle Egypt - Banysweif. *Orobanche* spp. on geranium (top) and dill (bottom).

Appendix 7

Activity I. Survey - Middle Egypt - Banysweif. *Orobanche* spp. on faba bean.

Appendix 8

A

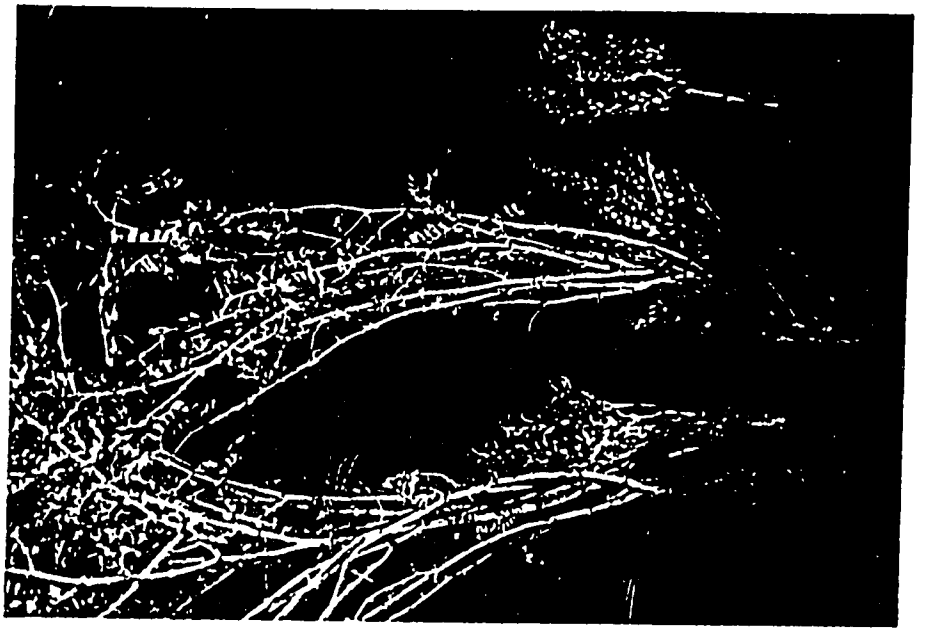


B

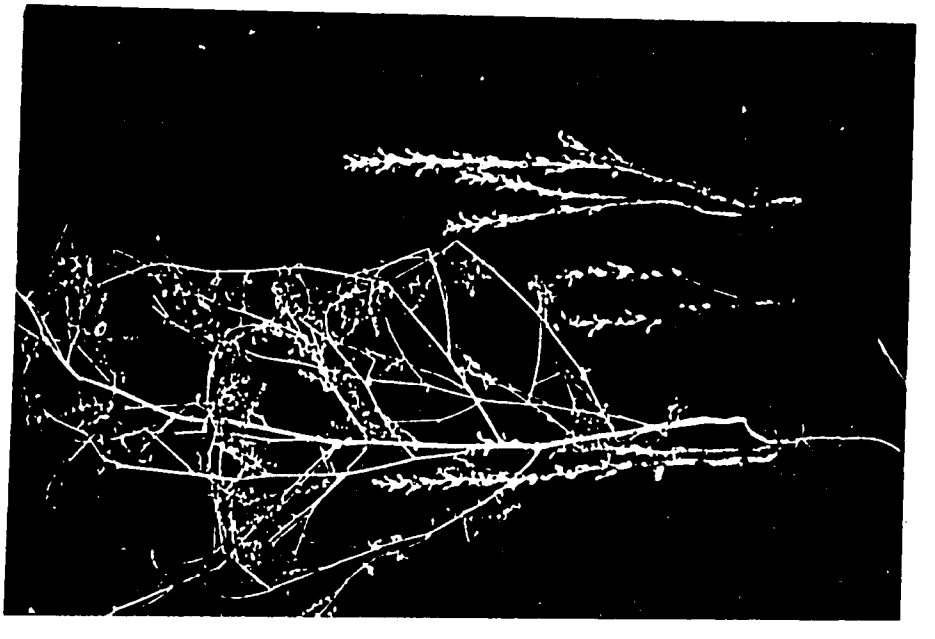
Activity I. Survey - Upper Egypt - Assuit. *Orobanche* spp. on Troapolium (A) and clover (B). The photos show how host/*Orobanche* sample are taken to the lab for registration (Activity I) and rhizosphere analyses (Activity III).

Appendix 9

A



B



Activity I. Survey - Suez Canal Region - Ismailia. *Orobanche* spp. on chick peas (A) and a weed (B).

Appendix 10

Activity III. *Orobanche* spp. in faba bean attacked by mites and fungi.

Appendix 11