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FINAL REPORT

PROJECT No. I-276-80

Studies on Broomrape (*Orobanche Spp.*) Biology and Control

R. Jacobsohn, C.L. Foy

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Tomatoes - Diseases and pests

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Studies on Broomrape (Orobanche spp.) Biology and Control

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הספרייה המרכזית
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C. ABSTRACT

Broomrape (Orobanche spp.) is a serious phanerogamic root parasite of many economically important broadleaf crops, mostly in semi-arid regions of the world. The heaviest losses in Israel and USA occur in tomato (Lycopersicon esculentum). Glyphosate is a potent systemic herbicide that has shown promise for selective control of broomrape in certain crops. A major screening program was conducted in Virginia (USA) to determine tomato varieties that have practical levels of tolerance to glyphosate. The same varieties were also tested in Israel for resistance to O. aegyptiaca.

The collection of the varieties included a wide range of genetic sources of the cultivated tomato (Lycopersicon esculentum) and representatives of 13 related wild species and subspecies. A total of 1522 tomato varieties were screened for glyphosate tolerance and about 190 tomato varieties showed fresh weights of treated plants 80% or higher of those of untreated plants. Statistical analysis of the data indicated that about 40 tomato varieties had fresh weights of treated plants not significantly different from those of untreated plants at a probability level of 80% or greater. All tomato varieties screened, however, showed injury to varying degrees by glyphosate applied at 37.5 g a.i./ha. Repeat screening of selected tomato varieties in the greenhouse and field indicated that some tomato varieties have promise for glyphosate tolerance and deserve attention in future screening programs. Autoradiography of ¹⁴C-glyphosate translocation indicated that radioactivity was translocated from tomato leaves to broomrape shoots without adversely affecting the host plants. A total of 1361 varieties including PUZ II were screened for resistance to O. aegyptiaca. No resistance was found. If there are minor

differences in susceptibility, the method of screening would not have discovered them. It is important to stress that the screening was done with a single source of Egyptian broomrape seeds that were collected from a tomato field in Ta'anch regions in the valley of Jezreel.

Improvements were developed in the method of infecting tomato plants behind glass in a soilless system by directly applying on the roots broomrape seeds that were pretreatment and stimulated to germinate using a synthetic germination stimulant. The problem of contamination was greatly solved by using a synthetic cloth instead of filter paper.

Further knowledge was obtained on broomrape seed germination in the laboratory. Results may suggest the use of broomrape seeds germination tests under extreme temperatures to distinguish between strains of the same species.

D. OBJECTIVES OF ORIGINAL RESEARCH PROPOSAL

The primary objectives of the present study are as follows:

- (1) to search for tomato varieties which have demonstrable (and practical) levels of tolerance to glyphosate (N-phosphonomethyl glycine), a potent systemic herbicide that has shown promise for selective control of broomrape in certain crops.
- (2) to screen tomato varieties that have demonstrable (and practical) levels of tolerance to broomrape.

If some tolerance to glyphosate and broomrape could be found in tomato varieties, an integrated control approach where genetic tolerance to the herbicide and the parasite can be combined through breeding programs could prove very useful.

The secondary objectives included:

- (3) Investigations on glyphosate a. transport, degradation and residue problems. This study, employing radiolabeled glyphosate, is an essential phase in the development of practical usage of glyphosate. Knowledge of the fate of the herbicide in the plant, particularly in relation to host plant development, is important from the scientific point of view as well as reflecting on practical aspects, such as the need for repeated applications. Residue information, is also required before any herbicide can be registered and introduced into agricultural practices.
- (4) To develop a method of rapid infection of tomato roots with Egyptian broomrape in a soilless system.

- (5) To improve broomrape seed germination. The last two objectives are mainly aimed to develop research methods. However, as various environmental parameters will be manipulated in both studies, we expect to obtain basic information concerning broomrape seed germination and root infection.
- (6) To study the effect of glyphosate on broomrape fine structure. This study is complementary to secondary objective a. They will provide considerable information on glyphosate mode of action.

The last secondary objective was drop according to the recommendation of the reviewing pannel. This recommendation was associates with a budget cut.

E. RESEARCH REPORT

INTRODUCTION

Broomrape (Orobanche spp.) is a parasitic herb, subsisting on the roots of broadleaf plants, mainly in areas with a dry and hot climate. The genus Orobanche consists of more than 150 species (Musselman, 1980), some of which are very host specific while others have a wide host range. Among the latter, most are serious parasites of economically important plants. The Middle East is widely infested with four of the most virulent species, namely, O. aegyptiaca Pers., O. crenata Forsk., O. cernua Leofl. and O. ramosa (or it's subspecies O. muteli Schulz). The various broomrapes are parasiting a wide range of crops which belong to the most ecomically important plant families such as Solanaceae, Umbelliferae, Cruciferae, Compositae, Cucurbitaceae and Papilionaceae (a legume family) (Jacobsohn 1984, Pieters 1979).

In Israel, broomrape has been known as an agricultural pest ever since modern agriculture was recorded. It was most likely a common plant in the old world flora. In recent years it has constantly encroached into new grounds and its various species are found in most parts of the country. Lately, the spread of broomrape has assumed alarming proportions and there are many farmers who are unable to find suitable land for growing susceptible crops. The most widely spread is the Egyptian broomrape (*O. aegyptiaca*). It is found on the Golan heights, lower and western Galilee, Jordan, Bet Shean and Arava Valleys. The valley of Jezreel, coastal plains, Northern and Western Negev. Crenate broomrape (*O. crenata*) is most common in the Bet Shean and Jezreel valleys and the Northern Negev (Shaar Hanegev). Nodding broomrape (*O. cernua*) is a prime concern in the Western and lower Galilee and the valley of Jerusalem sunflowers and tomatoes, and in the Jordan and Arava valleys on tomato and eggplant. Muteli broomrape (*O. muteli*) is a major problem of the potato seed production in the upper Golan heights. In addition, it is found alongside the Egyptian broomrape in several other location.

The broomrape problem in the United States involves two species. The first, *O. ramosa*, was first spotted in Kentucky in 1980, where it was seen parasitizing tobacco. Subsequently, this species of broomrape was found parasitizing tomatoes in California in 1929, where it reached the level of being a serious threat to the tomato industry by 1959. This triggered an eradication program using the very expensive method of soil fumigation with methyl bromide (Wilhelm, 1962). It has remained a minor but recurring problem since then. Recently, an infestation of *O. ramosa* has been discovered in south central Texas (Sand, 1981; Musselman and Nixon, 1981).

This infestation showed potential to parasitize tomato, tobacco, sunflower and other economically important crops (Eplee, 1984).

The second species of broomrape in the United States, O. minor, exists mostly in the eastern part of the country (Frost and Musselman, 1980). It is not considered a serious threat to any economically important crops in this country at present. However, O. minor has reached alarming proportions in New Zealand, where it has formed extensive infestations on clover to the extent of causing economic damage (Evans, 1962; James and Frater, 1977; Merry, 1947). It is viewed as a potential threat to the tobacco crop in that country. The lack of understanding of the population biology and behavior of this plant necessitates that it not be dismissed from consideration as a potential parasitic weed of leguminous crops and tobacco in the United States.

There are two stages in the host parasite relationship. First, the pre-infection stage that can be subdivided into the seed germination phase in which a germination tube is formed. Germination is induced by root exudates containing germination stimulants (Brown 1946). The second phase of the first stage is so far speculative in broomrape, namely, the germination tube is undergoing changes and transforming into an organ capable to attach itself to a root of a host plant (primary haustorium). The existence of this stage was proven in *Agalinis purpurea* (Riopel 1979). This phase is initiated by another compound of the host root exudates. The second stage, the parasitic stage can also be divided into two phases, first, initial contact and attachment of the primary haustorium. Rapid cell division occurs forming a spherical tubercle - haustorium.

The second phase follows by penetration into the host root tissue and creating contact with the host conductive tissues. Haustorium continues to

develop forming "roots" that may function as secondary haustoria and a primary bud that develops into the flowering stalk. Already at the stage of underground development, in which the parasite forms a sizeable biomass, considerable damage is incurred by the host, which signifies the need for an early control (Salle et al 1984).

Host/parasite relationship are more particular in the second stage than in the first. Several non-host plants are known to secrete broomrape germination stimulants (Brown et al., 1951) such as flax (Linum usitatissimum L.) (Chabrolin, 1935), corn (zea mays L.) and sorghum (sorghum sp.) (Brown et al., 1951). This discovery suggested the use of these plants as trap crops to reduce levels of infestation (Beilin, 1968; Brown, 1946; Kasasian, 1973b). The result of field experiments applying this principle were mostly negative (Davis, 1959) for reasons discussed elsewhere (Nash and Wilhelm, 1960; Jacobsohn and Foy, 1980).

Broomrape has been successfully germinated under laboratory conditions by several researchers from the mid-1930's (Brown et al., 1951; Chabrolin, 1935; Nash and Wilhelm, 1960). Germination was generally effected by means of root secretions from host and non-host plants on filter paper (Brown et al., 1951; Kasasian and Parker, 1971) or on agar (Abu-Shakra et al., 1970; Nash and Wilhelm, 1960; Ranga Swamy, 1963). Various plant growth regulators and other compounds have also been investigated as stimulants of broomrape seed germination (Garas, N.A. 1974). A series of synthetic broomrape seeds (and Striga) germination stimulants (GR-7, GR-24, GR-45 and others) were synthesized by A.W. Johnson, A.W. 1976). Those compounds are successfully used in research. So far, in spite of considerable efforts invested, scientists failed to isolate and characterize the natural broomrape seed germination stimulant.

In isolated cases, germination was spontaneous without the aid of stimulants.

As already mentioned, considerable damage is caused by the host plant prior to the emergence of the broomrape plants above the soil. The most pronounced damage is caused during the rapid elongation of the inflorescence stem, associated with diversion of essential nutrients from the host plant to build the 'body' of the parasite plant. When infestation is severe, the host plants are liable to collapse at this stage. Even when the plants do not actually die, the economic damage is reduced yield is likely to be severe.

The effective control of broomrape is beset with numerous difficulties. Considerable damage is incurred by the economic crop, even before the emergence of the parasite, which only triggers the farmer's awareness of the problem. The seed production potential of broomrape is tremendous. Its seeds are small (0.2-0.3 mm long) and each plant is capable of producing thousands of seeds. The seeds will generally not germinate unless they are found in the rhizosphere of host plants or non-host plants which secrete suitable germination stimulants. The seeds remain viable in the soil for a very long period. These characteristics foster the accumulation of vast stores of seeds in the soil so that crop rotation, as a mean of reducing the inoculum in the soil, is not efficient.

Several methods have been employed for the control of broomrape. Some of the earlier methods included hand weeding. This method is labor intensive and ineffective because, as mentioned, damage occurred prior to emergence and new broomrape plants continue to appear as long the host roots continue to grow.

Controlling broomrape by means of mechanized cultivation is also not feasible since most of the parasite plants appear within the crop row. Furthermore, there appear to be definite limitations to the trap crop method for reducing the amount of inoculum in the soil, as already discussed above. Controlling the parasite by solar heating of the soil was described by Jacobsohn, et al. (1980). Its main disadvantage is the high cost of the polyethylene needed to mulch the soil.

Biological control of broomrape has been attempted mainly in eastern Europe and USSR by means of the insect Phytomyza orobanchia Kalt., which feeds on the inflorescence of the parasite (Klyueva and Pamukchi, 1978; Lekic, 1974; Nemli and Giray, 1983; (Pamukchi, 1979), Sushchinski, 1969). In a large scale experiment in the USSR, Phytomyza orobanchia larvae distribution in the field resulted in killing more than 50% of broomrape plants and preventing the rest from setting seed (Klyueva and Pamukchi, 1982). In small plot field trials, Fusarium solani and the most aggressive F. oxysporum have been observed to effectively control O. ramosa in tomatoes without adversely affecting the crop; (Pamukchi, 1979). In certain soils, Rhizoctonia solani has been detected as the main factor in suppressing the growth of broomrape in tomatoes (Gold et al., 1979). Although some success has been achieved in obtaining biological control of broomrape, particularly in the USSR, this method is unlikely to provide broomrape control on a broader scale in the near future (Girling et al., 1979).

Another approach to attacking the broomrape problem is the development of resistant cultivars of susceptible crops. Resistant cultivars of sunflowers have been known since the 1930's (Beilin, 1968). Tolerance to various degrees has also been reported for eggplants (Solanum melongena L.)

(Dalela and Mathur, 1971a) mustard (Brassica sp.) (Dalela and Mathur, 1971b), broad beans (Vicia faba L. var. major L.) (Cubero, 1973; Hernandez et al., 1984) and vetch (Vicia sativa L.) (Gil et al., 1984). Thus far, the systematic development and use of new resistant crop cultivars has been limited.

Considerable research effort has been devoted the finding selective chemical control methods for broomrape (Pieterse, 1974). These approaches include (a) treatments to attack the broomrape directly, (b) treatment of the host plant to prevent or impair parasitic development and (c) application of synthetic stimulants to induce suicidal germination of broomrape (Johnson et al., 1976, Jacobsohn et al., 1978). Generally, soil fumigants (especially methyl bromide) applied prior to planting have been effective (Rogers, 1972; Wilhelm et al., 1976; Zahran, 1970). In many instances, however, their use is prohibitively expensive.

Literally hundreds of "promising" preplant soil-incorporated, preemergence and postemergence herbicides have also been investigated (e.g. Kasasian and Parker, 1971; Saghir et al., 1972; Lange et al., 1975; 1979).

Results in advanced field testing, however, have been largely disappointing, indicating the intricate relationship that exist among the host, parasite, environmental conditions and herbicide.

Of all herbicides tested, glyphosate, used as a systematic postemergence spray to the host crop, currently shows most promise for widescale development. Glyphosate is a broad spectrum, foliarly applied herbicide which is relatively non-selective (Beste et al., 1983) nevertheless, some interesting selectivities have been demonstrated in certain crops, including some that are attacked by broomrape (Kasasian, 1973a; b; Lange et al., 1975; 1976; 1979; Petzoldt, 1979; Saghir, 1979;

Schluter and Aber, 1979; Schmitt et al., 1979; Jacobsohn and Kelman, 1980; Kukula and Masri, 1984). Glyphosate is readily absorbed and translocated throughout the treated plants (Gianfagna, 1975; Sprankle et al., 1975) and may transfer systemically and accumulate in the attached parasite. Some field studies have shown that when glyphosate is applied at considerably low rates (60 to 120 g/ha), it can suppress and/or control the attached broomrape possibly by translocation from the host to the parasite (Kasasian, 1973; Lange et al., 1975; 1976; Petzoldt, 1979; Saghir, 1979; Schluter and Aber, 1979; Jacobsohn and Kelman, 1980).

The effectiveness of glyphosate as a foliar application for O. crenata control in broad beans (*Vicia faba* L.) was first reported by Kasasian (1973). The rate of 200 g/ha provided complete control of the parasite and sufficient safety margin, indicating the relative resistance of broad beans to the herbicide. Similar results were obtained with O. aegyptiaca control in tobacco (Kasasian, 1973). Several researchers have since confirmed that glyphosate could be used for selective control of broomrape in crops such as broad beans and tobacco (Jacobsohn and Kelman, 1980; Schmitt et al., 1979; Schluter and Aber, 1979). The results of selective control of broomrape with glyphosate in some other crops, such as tomato carrots and peas, however, have been less encouraging. Rates of the herbicide as low as 50 to 100 g/ha were observed to control O. aegyptiaca growing on tomatoes but the treatments caused injury on crop plants (Jacobsohn and Kelman, 1980). Hence, the limiting factor to the use of glyphosate for broomrape control is the margin of herbicide selectivity in these crops.

Tomato is one of the most important vegetable crops in Israel, the United States and worldwide. All the known cultivated varieties of tomato

are highly susceptible to broomrape, especially to O. aegyptiaca and O. ramosa. Of over 100 tomato varieties tested in the greenhouse for resistance to the root knot nematode (Meloidogyne incognita and M. javanica), tomato yellow leaf curl virus, and Orobancha in Jordan, about eight tomato varieties showed slight tolerance to Orobancha (Abu-Gharbieh et al., 1978).

The primary objectives of the present study are as follows: (a) to search for tomato varieties which have demonstrable (and practical) levels of tolerance to glyphosate (N-phosphonomethyl glycine), a potent systemic herbicide that has shown promise for selective control of broomrape in certain crops, and (b) to screen tomato varieties that have demonstrable (and practical) levels of tolerance to broomrape.

If some tolerance to glyphosate and broomrape could be found in tomato varieties, and integrated control approach where genetic tolerance to the herbicide and the parasite can be combined through breeding programs could prove very useful.

The secondary objectives includes:

(a) Investigations on glyphosate transport, degradation and residue problems. This study, employing radiolabeled glyphosate, is an essential phase in the development of practical usage of glyphosate. Knowledge of the fate of the herbicide in the plant, particularly in relation to host plant development, is important from the scientific point of view as well as reflecting on practical aspects, such as the need for repeated applications. Residue information is also required before any herbicide can be registered and introduced into agricultural practices.

(b) To develop a rapid method of infecting tomato roots with Egyptian broomrape in a soilless system.

(c) To improve broomrape seed germination.

The last two objectives are mainly aimed to develop research methods. However, as various environmental parameters will be manipulated in both studies, we expect to obtain basic information concerning broomrape seed germination and root infection.

MATERIALS AND METHODS

Screening Program for Glyphosate Tolerance - Preliminary Experiments (US)

Preliminary experiments using two varieties of tomatoes, Glamour (determinant) and Westover (indeterminant), were conducted in the greenhouse. Tomato plants were grown in 15-cm diameter pots filled with a potting mix containing Weblite (40%), vermiculite (40%) and peat moss (20%). Lime (42.5 g), 4-9-3 fertilizer (80.0 g), and Osmocote, a slow release 14-14-14 fertilizer (85 g), were added to each 0.028 cubic meter of the potting mix. Six rates of glyphosate ranging from 0 to 200 g/ha were applied in 250 l/ha using a knapsack sprayer equipped with a single 8002 E nozzle. Two applications of each rate were made as follows: (1) tomato plants 6 and 8 weeks old, (2) tomato plants 8 and 10 weeks old, (3) tomato plants 10 and 12 weeks old. Tomato plants were harvested approximately 4 weeks after the second application of glyphosate in each case and plant response was evaluated by plant height measurements, plant fresh weights, and the number and fresh weight of fruits. All treatments were replicated six times and the experiments were conducted three times.

In subsequent experiments, younger tomato plants (cv. Glamour) were employed because of the large number of tomato varieties scheduled for screening and the space required (as well as other considerations for larger plants). Plants were grown in 7.62 cm diameter pots filled with the same potting mix as used in earlier experiments. Eight rates of glyphosate ranging from 0 to 150 g/ha were applied in 250 l/ha of water to tomato

plants at the two-true-leaf stage by means of a compressed air, continuous moving belt laboratory sprayer. Treatments were replicated six times and experiments were conducted twice. Plant response was evaluated by determining plant fresh weights 12 to 16 days after treatment.

Determination of Spray Volume for Glyphosate Application on Tomatoes (US)

Tomato plants (cv. Rutgers) were grown in 7.62 cm diameter pots in the greenhouse. The pots, each containing one or two plants, were arranged in pairs based on the number and the size of the tomato plants. When the plants reached the two-leaf stage of growth, one pot of each pair was sprayed with glyphosate and the other was left untreated. Six rates of glyphosate (12.5 to 100.0 g/ha) were tested and each rate was applied in spray volumes of 93, 197, 384, 561, and 748 l/ha. Response of tomato plants to various rates of glyphosate and spray volumes was determined by comparing fresh weights of treated plants with those of untreated plants from each pair.

Screening of Tomato Varieties for Glyphosate Tolerance in The Greenhouse (US)

Tomato varieties, obtained from the USDA Regional Plant Introduction Station, IOWA State University, Ames, Iowa, and University of California, Davis, California, USA, were grown in 7.62 cm diameter plastic pots in the greenhouse. When the plants reached the 2 to 3 leaf stage, the pots, each containing one or two plants, were arranged in pairs. The pairing was done on the basis of the number and size of tomato plants. One pot of each pair was treated with glyphosate at 37.5 g/ha in 250 l/ha spray volume. Ten or fewer pairs, each pair representing one replicate, were used for each tomato variety. The plants were harvested by clipping the stem at soil

level and their shoot fresh weights determined 12 to 16 days after treatment. Fresh weights of treated plants were converted to a percent of the fresh weight of untreated plants. The mean percent difference in untreated and treated plant fresh weights was subjected to statistical analysis by using the t-test.

Re-evaluation of Some Tomato Varieties for Glyphosate Tolerance in the Greenhouse (US)

Tomato varieties with the fresh weights of treated plants equal to 80% or greater than those of untreated plants in the main screening program were selected for re-evaluation for glyphosate tolerance in the greenhouse. These varieties were selected from the 1022 tomato varieties screened for glyphosate tolerance up to the end of 1983. The plants belonging to 56 tomato varieties were planted in June, 1984 in 7.62 cm diameter plastic pots. The potting medium used in this experiment was the same as that used in the preliminary experiments and in the main screening program, i.e. Spasoff mix (Weblite (50%), vermiculite (40%) and peat moss (20%) with lime and fertilizer added). When the plants reached the 2 to 3 leaf stage, the pots, each containing one or two plants, were arranged in pairs on the basis of number and size of plants. As in the case of the main screening program, one pot of each pair was treated with glyphosate at 37.5 g/ha in 250 l/ha spray volume. There were ten or fewer pairs or replicates for each tomato variety. Shoot fresh weights from each pot were obtained 15 days after treatment. The data were converted into mean percent difference between untreated and treated plant fresh weights for each variety and analyzed using the t-test.

Evaluation of Some Tomato Varieties for Glyphosate Tolerance in the Field (US)

Tomato varieties selected from the main screening program and reevaluated in the greenhouse were also evaluated in the field for glyphosate tolerance. Only 39 tomato varieties were planted in the field during August, 1984 due to limitations of space and manual help. Tomato plants were grown in the greenhouse for 21 days and transplanted in the field in rows. Thirty of the 39 tomato varieties selected were planted in one field and the other nine were planted in a second field. There were two rows in one replication and each row contained one plant each of the 30 tomato varieties. There were ten plants of each of the nine varieties in each row. There were five replications in each experiment. About 14 days after transplanting (8 to 10 leaf stage of plants), one row in each replication was sprayed with glyphosate at 60 g/ha in a spray volume equivalent to 250 l/ha with a CO₂ knapsack sprayer. Precaution was taken to avoid spray drift from reaching the adjacent untreated rows.

The plants in the experiment with 30 tomato varieties were allowed to grown up to maturity before they were harvested. At harvest, observations were recorded on plant height, plant vigor, shoot diameter, shoot fresh weight and fruit fresh weight. The plants in the experiment with only nine tomato varieties were harvested within 21 days of treatment. Observations on these varieties were recorded on the diameter of the injury symptoms in the terminal shoot meristem, vigor of treated plants relative to that of untreated plants, and on the dry weight of untreated and treated plants. The data from both experiments were converted to mean percent difference between untreated and treated plants where appropriate and analyzed by using the t-test.

Evaluation of some tomato varieties for glyphosate tolerance in the field, (Israel).

About 50 of the relatively glyphosate tolerant varieties were sent to Israel during early 1984. Due to space limitation at the experiment station near Acre, we selected the 15 most tolerant varieties according to the data available at that time. Those varieties were evaluated in a broomrape infested field.

The experiment was planned in a split-plot design to have three replication. Each plot for each variety consisted of a methyle bromide fumigated subplot (broomrape free) (12 m long and 1.8 m wide) which in turn was again subdivided into three sub-subplot of 4 meters each to accomodate three glyphosate treatments of 0, 50 and 100 gr/ha in a spraying volume of 300 leters. Each sub-subplot was planted with 12 tomato plants.

The seeds were planted in speedling trays on April 5th 1984 and transferred to the field on May 10th. The field was sprayed on June 4th. On June 18th plant height was determined (three measurement in each sub-subplot-spraying rate). On July 3rd a visual evaluation of glyphosate damage was conducted. The visual evaluation took into account growth retardation, yellowing of the leaves and spindliness (formation of small spile-like leaves). Until July, 15 no broomrape emerged, and non was found on the roots. Therefore, we decided not proceed with yield harvest.

Screening tomato varieties for resistance to Egyptian Broomrape -Preliminary Experiment (Israel)

Preliminary experiments to determine a proper potting soil mixture and rate of artificial infestation were conducted in the field in the summer of 1981. Various ratios of clay soil sand, tuff and peat were teste in two

liter plastic pots that were buried in the field to the depth of soil level inside the pot.

Infestation levels of 25, 50 and 100 mg of dry Egyptian broomrape seeds per pot were also tested.

The project plan was to screen a considerable number of tomato varieties with at least 6 replication. Therefore, counting broomrape infections seemed to be impractical and not necessary for initial screening. Instead, the method of visual observation was adapted. Counts of broomrape plants were planned only if there were less than 20 infections present.

Screening tomato varieties for resistance to Egyptian Broomrape (Israel)

First year spring 1982

The experiment was conducted at the experiment station near Acre, 530 tomato varieties were available. The plants were initiated in pairs in "speedling" trays on March 12, 1982 in the greenhouse. Pairs of plants were transplanted into 2 liter pots in the field on April 26. The pots were placed into the ground to a depth approximately equal to the soil level inside the pot. Evaluation of broomrape infection was done on June 9, 1982. The evaluation was done by dumping the pot content and shaking the soil off the roots. The number of broomrape attachments was visually graded on a 1 to 3 scale (3 very heavy infection). The experiments were replicated six times.

Second experiment - spring 1983

The experiment was conducted in the field of the settlement of Shave-Zion in the Western Galilee. The experiment was designed in exactly the

same way as in the previous year. The first shipment of seeds for the second year experiment included 235 varieties of which 221 varieties were new and 14 varieties that had low infection in the first year trial were retested. These seeds were planted in speedling trays on March 25, 1983 in the greenhouse and were planted in pots in the field on May 5.

The second shipment of seeds arrived a few weeks later and includes 495 varieties. Those seed were started in the greenhouse on April 28 and were transplanted into pots in the field on May 30.

Third experiment - spring 1984

This experiment conducted in the greenhouse at Bet Dagan. Small plastic pots of about 6³cm were filled with potting mixture containing 30 mg of broomrape seeds per 1 kg of soil. Several tomato seeds were placed in each pot and eventually thined to two plants per pot. 703 varietie, received from the Tomato genetics stock center, University of California, Davis, were tested in the experiment. The experiment was replicated six times and was started on February 16. Broomrape infection was evaluated from April 10 to 16.

Ten varieties were found free of broomrape infection. Those plants were replanted in two liter pots with infested soil. The pots were placed in the field. The tomato plants were grown to maturity and seeds were produced from the fruit.

Screening Tomato Varieties for Resistance to Egyptian Broomrane - confirmation Experiment (Israel).

The experiment was conducted in the winter of 1984/85. Seeds of the 10 varieties were planted in 10 cm (diameter) pots which contained potting soil infested with 0.2 mg (340 seeds) and 6 mg (1000 seeds) per pot. The experiment was started December 5, 1984 in the greenhouse and terminated on February 12-13.

Effect of Soil Mixtures on Broomrape Infestation on Tomatoes in the Greenhouse (US).

Three types of soil mixtures were tested for their effect on broomrape infestation on tomato plants in the greenhouse. The types of soil mixtures used were (A) clay loam (33.3%), sand (33.3%), and weblite (33.3%); (B) clay loam (45%), and peat moss (10%), and (C) vermiculite (40%), weblite (40%), and peat moss (20%). Tomato seeds belonging to cvs. 'Rutgers' and 'PUZ II' were planted in trays containing 'Spasoff' mix (webite, 40%; vermiculite, 40%; and peat moss, 20%). When the plants reached the first-true-leaf stage, they were transplanted in 15 cm diameter plastic pots containing the three types of soil mixtures. There were 12 pots for each soil mix and each cultivar, and each pot contained two plants. At the time of transplanting, 5 mg of O. aegyptiaca seeds were dispersed in the root zone of each tomato plant. All necessary precautions were taken to ensure that broomrape seeds do not spread to regions beyond the experiment.

Observations were recorded on the emergence of broomrape plants above the soil surface. When most of the broomrape plants had emerged, tomato plants were carefully removed from the pots and the soil was gently washed off from the roots and the attached broomrape plants. Observations on broomrape were recorded on the number of infections per plant, the mean length and fresh weight of broomrape shoots. Observations on tomato plants were recorded on shoot height, shoot fresh weight and root fresh weight. The number of plants for each observation ranged from four to 12. The data was analyzed statistically and the means were compared using the least significant difference at the 5% level of significance.

Translocation of ^{14}C -Glyphosate in Broomrape through the Host (US)

Tomato plants (cvs. Rutgers and PUZ II) were grown and infected with broomrape (*O. aegyptiaca*) in a manner similar to that described in the above experiment. The infected tomato plants were used for investigating the translocation of ^{14}C -glyphosate from host leaves to broomrape shoots.

^{14}C -Glyphosate (specific activity 1.97 mCi/mmole) labeled on the carbon atom adjacent to the phosphorus was supplied as the parent acid and was converted to the monoisopropylamine salt by the addition of isopropylamine in a 1:1 molecular ratio. Tomato plants were treated on 12 leaflets of four mature leaves with a 10 μl droplet of ^{14}C -glyphosate containing 0.021 $\mu\text{Ci}/10\mu\text{l}$ (total dose per plant 0.25. μCi). All plants were in the 4 to 5 true leaf stage of growth at the time of treatment. Each plant received approximately 0.02 mg glyphosate and there were 20 plants for each of the two varieties of tomato.

Comparative uptake and translocation of ^{14}C -glyphosate was investigated using the gross autoradiographic methods of Crafts and Yamaguchi (1964). Half of the treated plants were harvested three days after treatment and the rest of the plants were harvested seven days after treatment by carefully removing them from the pots and washing off the soil by water. The excess moisture was removed from the roots with absorbant paper towels. The plants were then sectioned into the treated leaves, the growing point and the roots with broomrape and mounted, pressed for 24 hours, and oven-dried for 48 hours at 70°C . The dried mounts were then exposed to GAF x-ray film for 16 days, at which time the film was developed and examined for ^{14}C translocation.

Infecting tomato plants with Broomrape in a Soilless System (Israel)

Developing the method was started prior to the initiation of the BARD project. The subject was adapted into the research proposal for further development. For the purpose of completion, earlier work will also be mentioned. The experimental unit consists of a filter paper sheet (16 x 10 cm) placed behind a glass plate (20 x 10 cm). The filter paper is supported by a 1 cm thick rubber foam layer (13 x 10 cm) (to allow aeration) and another glass plate of the same size as the rubber foam. The whole unit is bound together with two rubber bands. All the components were autoclaved prior to assembling. Young plantlets are prepared by germinating seeds in a roll of germinating paper. The seeds are surface sterilized with sodium hypochlorite (2% active chlorine for 5 minutes) germinating the seeds in a vertically placed roll provide plantlets with straight roots. After about 7-8 days (temp. 30°C day, 20°C night, tomato plantlets with erect cotyledons and a root of 5-8 cm long are planted between the glass plate and the filter paper, and then, surface sterilized broomrape seeds are sprinkled over the paper. Finally, a sheet of black polyethylene - 13 cm wide, is wrapped around the unit to darken the area of the roots, and the planted unit is placed into a beaker or a tray with 2 cm deep nutrient solution. The solution is moving up the paper by capilarity.

The method suffered from two problems.

1. Little uniformity of infection was found between the units.
2. The filter paper was rapidly contaminated. The whole operation was done on a sterile banch (laminar flow) but afterwards the units are placed in a non sterile environment. In order to improve the method, the following was tried.

- (a) Replace the whatman no.1 filter paper by a synthetic cloth.
- (b) Precondition the seed on 10 mm wet fiber glass, filter paper discs for ten days and than apply the seeds directly on the roots, by lightly rubbing the discs on them.
- (c) Expose the seed for 24 hours prior to application on the roots to a synthetic germination stimulant (GR24).

a. Replacing the Whatman no. 1 filter paper by a synthetic cloth

The cloth that was used was relatively thin, made of a multifiber thread and coarsely woven. The experiment started October 7, 1984 and was terminated Nov. 15, 1984. The experiment was conducted in a growth chamber at 25°C and a photoperiod of 14 hours.

b. Preconditioning the seeds and stimulating them to germinate

The glass growing units were prepared with synthetic cloth and planted with 4 tomato seedlings on December 3, 1984. About 400 egyptian broomrape seeds were applied to each unit. Half of the units received preconditioned seeds (kept wet for 10 days) and the other half received preconditioned seeds that were also treated for two days with a synthetic stimulant (1 ppm of GR 24). The nutrient solution was 1/4 concentration of Hoagland solution.

Improving Broomrape Seed Germination in The Laboratory (Israel)

Following is a description of the method of broomrape seed germination.

a. Wetting the seeds. Dry broomrape seeds are stirred in water + 0.1% of surfactant on a magnetic stirrer for 15 minutes in order to thoroughly wet the seeds.

b. Surface sterilization

Seeds are transferred into a sodium hypochlorite solution containing 2% active chlorine and 0.1% surfactant for 5 minutes. The seeds are then thoroughly washed with sterilized water and dried overnight on the filter paper used in the buchner funnel and covered with filter paper.

c. Pre-treatment of the seeds:

Discs. 10 mm in diameter, made of fiber-glass filter paper are placed on a regular filter paper in a petridish.

About 20 dry broomrape seeds are scattered on each disc, and finally sterilized water is added to the extent of thorough wetting of the filter paper and the discs. The petridish with seeds are placed at 23-25°C for ten days.

The wet discs with the seeds are removed from the petridish, blotted on a dry filter paper and then transferred to another petridish. 30 microliters of a germination stimulant (concentration of 1 ppm of GR 24) is added to each disc. The petridish is placed again at 20-25°C germination is counted after 5 days.

Several aspects of the germination method were studied.

Seed disinfection

Several disinfection agents were tested, namely sodium hypochlorite at concentrations of 1%, 3% and 5% of active chlorine, calcium hypochlorite at concentration of 1%, 2% and 3% active chlorine and Ethanol concentrations

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of 50%, 70% and 90%. Exposure times of the broomrape seeds to each of the agents were 1 minute and 5 minutes.

Seed washing

Different amount of sterilized water were used to wash the seed on the buckner funnel after removal of the sodium hypochlorite (Table) seed of nodding broomrape (*O. cernua*) were used in the experiments.

Pre-treatment period

The necessary length for achieving maximum seed germination was studied with the four broomrape species previously mentioned. Seed samples were removed daily for 18 days and treated with a synthetic germination stimulant (GR 24) and than placed at 25°C to germinate.

Temperature effect

The usual germination procedure was used. The seed were exposed to the various temperatures in both pre-treatment stage and germination stage. The various experiments were conducted from mid June to late July 1984.

RESULTS AND DISCUSSION

Screening program for Glyphosate Tolerance Preliminary Experiments (US)

Two varieties of tomatoes, Glamour and Westover, were tested in the greenhouse for their tolerance to low rates of glyphosate. Results of these experiments indicated that the stage of growth of tomato plants affected their tolerance to glyphosate. The fresh weight of tomato plants (cv. Westover) sprayed twice at 10 and 12 weeks after planting with glyphosate at 200 g/ha was 90% of that of untreated plants. The fresh weight of tomato plants sprayed at 6 and 8 weeks after planting, however, was only 8% of that of untreated plants. Similar results were observed with cv. Glamour, although this variety appeared to be slightly more susceptible to glyphosate than cv. Westover. When the plants were sprayed 6 and 8 weeks or 8 and 10 weeks after planting, very few fruits were produced. When the plants were sprayed at 10 and 12 weeks after planting with glyphosate at 200 g/ha, however, fresh weights of fruits on treated plants ranged from 60 to 90% of those on the untreated plants.

In subsequent experiments, younger tomato plants (2 to 3 weeks old, cv. Glamour) at their second true-leaf stage were sprayed with various rates of glyphosate ranging from 0 to 150 g/ha in spray volume equivalent to 250 l/ha. From the fresh weight of tomato plants obtained 12 to 16 days after treatment, a response curve was plotted. From this response curve, 37.5 g/ha rate of glyphosate was chosen for the final screening program. Plants in the field will not be sprayed at such an early stage; however, plants as young as the 4 to 5 leaf stage have been found to be infected with broomrape.

Determination of Spray Volume for Glyphosate Application on Tomatoes (US)

Tomato plants (cv. Rutgers) were sprayed at the 2-leaf stage with various rates of glyphosate (12.5 to 100 g/ha) in five different spray volumes in the greenhouse. The tolerance of tomato plants to glyphosate at 50.0, 67.5 and 100 g/ha increased as the spray volumes increased from 93 to 748 l/ha (Table 1). At lower rates of the herbicide (12.5 to 25.0 g/ha), a similar trend of increased tolerance was apparent as the spray volume increased from 93 to 187 l/ha. With spray volumes over 187 l/ha, however, differences in tolerance of tomato plants to glyphosate were not clearly evident. A spray volume of 250 l/ha was considered to be most appropriate for screening tomato varieties for glyphosate tolerance in the greenhouse.

TABLE 1. EFFECT OF SPRAY VOLUME ON TOXICITY OF GLYPHOSATE TO TOMATO PLANTS (VARIETY RUTGERS)

Rate of glyphosate (g/ha)	Tomato fresh weight (% of untreated)				
	Spray volume (l/ha)				
	93	187	374	561	748
12.5	72.4	90.4	76.4	71.8	100.4
18.9	50.0	82.6	84.0	84.2	79.4
25.0	46.8	80.6	81.2	94.4	71.8
50.0	15.6	41.2 *	67.0	85.4	70.8
67.5	15.0	36.2	44.0	58.4	85.2
100.0	11.8	19.6	24.5	42.2	51.8

* Glyphosate at 37.5 g/ha was applied in 250 l/ha of water in the major screening program.

These results indicated that by increasing the spray volume for glyphosate application at a given rate, the injury to tomato plants from the herbicide can be reduced.

Screening of Tomato Varieties for Glyphosate Tolerance in The Greenhouse (US)

The main screening. A total of 1522 tomato varieties were screened in the greenhouse to evaluate their tolerance to glyphosate at 37.5 g/ha applied in a spray volume equivalent to 250 l/ha. Out of these 1522 tomato varieties, 108 tomato varieties were screened during 1980 and 1414 tomato varieties were screened between March, 1982 and April, 1985. Fresh weights of treated and untreated plants for each variety are presented in the Appendix. See last page.

All tomato varieties included in this screening program were injured to varying degrees by glyphosate application. All the treated plants showed typical injury symptoms induced by glyphosate on susceptible plants. These injury symptoms included retardation of growth and chlorosis in the meristematic regions of the shoot which appeared within one week of treatment (Figure 1). Plants that were able to overcome the initial inhibition of Growth developed thin, spike-like leaves. Plants of some tomato varieties, however, were able to completely overcome the initial injury from glyphosate and resumed normal growth.

Fresh weight of tomato varieties treated with glyphosate showed a wide range of differences from those of control plants (Figure 2). About 1330 tomato varieties showed more than 20% reduction, 860 tomato varieties showed more than 40% reduction and 630 tomato varieties showed more than 50% reduction in the fresh weight of treated plants. About 151 tomato varieties showed fresh weights of treated plants between 80 and 100% of

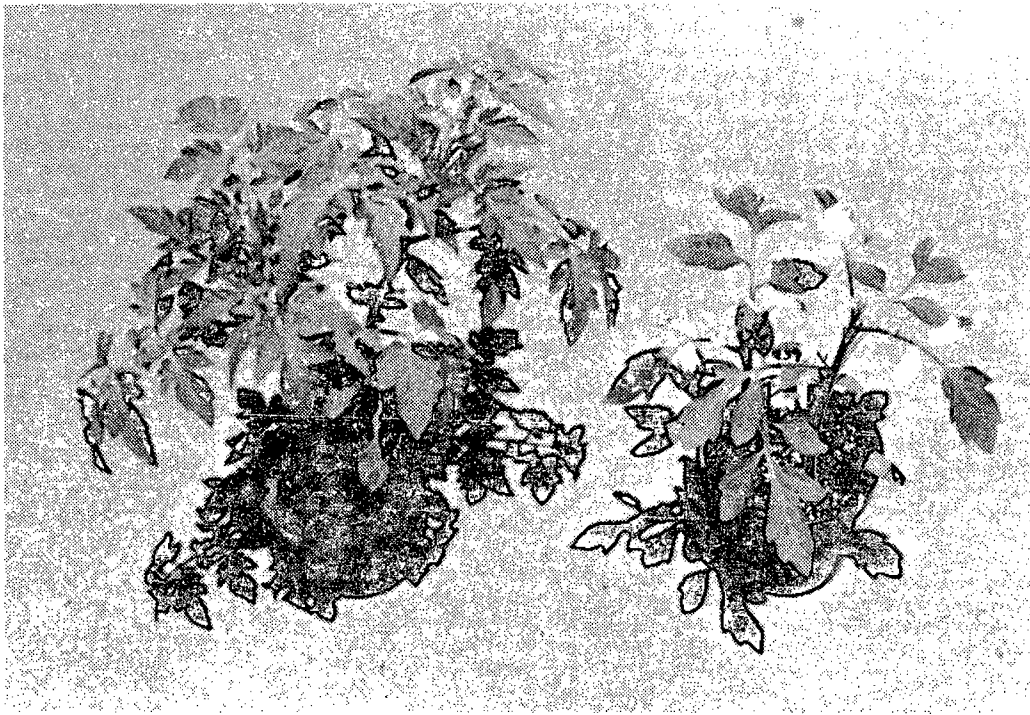
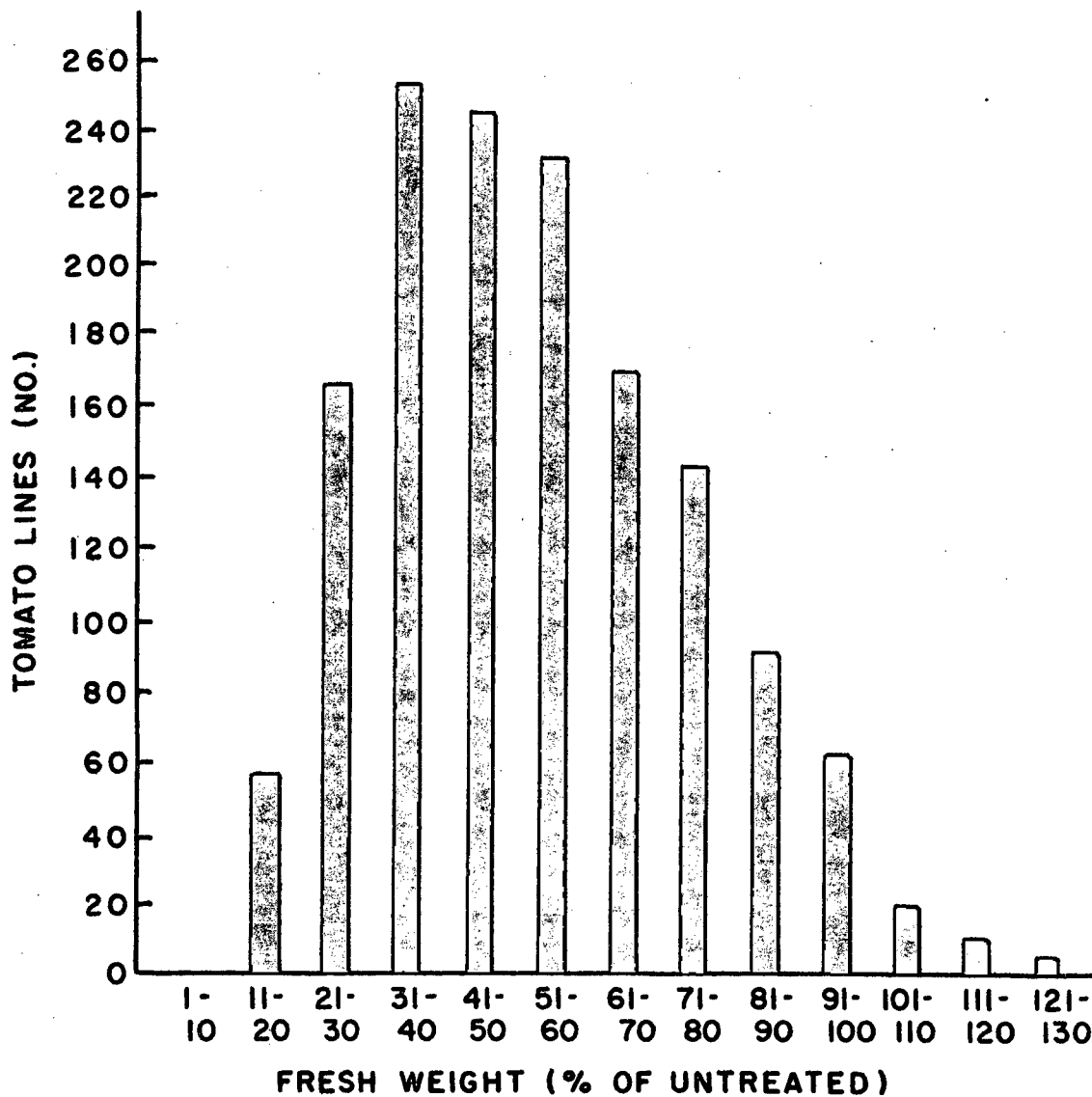


Figure 1. The untreated tomato plant and glyphosate (37.5 g/ha) treated tomato plant showing the injury symptoms.

Figure 2. Tolerance of tomato varieties to glyphosate at 37.5 g/ha.



those of control plants and about 41 tomato varieties had fresh weights of treated plants higher than those of control plants.

Statistical analysis conducted on the mean percent differences between the fresh weights of untreated and treated tomato plants indicated that 74 tomato varieties out of all the varieties screened had no significant effect of glyphosate on fresh weights at a probability level greater than or equal to 50% ($P \geq 0.5$). Out of these 74 varieties, the fresh weights of 34 tomato varieties were not significantly affected by glyphosate at a probability level between 50 and 80% ($P \geq 0.5$ and $P < 0.8$). The fresh weights of 20 tomato varieties were not significantly affected at a probability level between 80 and 95% ($P \geq 0.8$ and $P \leq 0.95$), and the fresh weights of the rest of the 20 tomato varieties were not significantly affected by glyphosate at a probability level of 95% or greater ($P \leq 0.95$). Since the t-test takes into account the deviation of means of treated plants around that of the mean of untreated plants (100%) for calculating significance levels, the 16 tomato varieties with mean fresh weights of treated plants exceeding those of untreated plants by at least 10% were included in the 20 tomato varieties that showed no significant effect of glyphosate at a probability level of 95% or greater. The fresh weights of treated plants of all 74 tomato varieties were greater than 35% of those of control plants.

It has been indicated that some tomato varieties show tolerance to 2,4-D (Appendix). From our results, it appears that there is no correlation between tolerance of tomato varieties to 2,4-D and tolerance to glyphosate.

Reevaluation of Some Tomato Varieties for Glyphosate Tolerance in the Greenhouse (US).

Fifty six tomato varieties were selected for reevaluation of their tolerance to glyphosate in the greenhouse. All these varieties had the fresh weights of treated plants 80% or greater than those of untreated plants in the primary screening program. Out of these 56 varieties, 41 tomato varieties showed fresh weights of treated plants lower than 80%, 31 tomato varieties showed fresh weights of treated plants lower than 60% and 26 tomato varieties showed fresh weights of treated plants lower than 50% of those of untreated plants upon reevaluation (Table 2). Only 15 tomato varieties had fresh weights of treated plants greater than 80% and only 5 tomato varieties had fresh weights of treated plants greater than 100% of those of untreated plants.

Statistical analysis of the data showed that out of all the 56 varieties tested, only 4 tomato varieties showed no significant difference in fresh weights of treated and untreated plants at a probability level at 50% or greater ($P \geq .05$). Out of these 4 tomato varieties, only two tomato varieties (labels 964 and 1149) showed no significant difference between fresh weights of treated and untreated plants at a probability level of 80% ($P \geq 0.8$) or greater and only one tomato variety (label 964) showed no significant difference in fresh weights of treated and untreated plants at a probability level greater than 95% ($P \geq 0.95$).

The difference in the susceptibility of tomato varieties in the main screening and the repeat experiment could be due to the differences in growth stage of plants at the time of treatment. It has been seen in preliminary experiments that the tolerance of tomato plants varies considerably with the growth stage of plants at the time of treatment.

LEGENDS FOR TABLE 2

- LABEL = Code numbers given to tomato varieties at VPI&SU, Blacksburg, Virginia.
- SCINAME = Scientific code name of tomato varieties.
- LYCES = Lycopersicon esculentum
- LYCPI = Lycopersicon pimpinellifolium
- ES*PI = L. esculentum X L. pimpinellifolium
- LYCPE = Lycopersicon peruvianum
- LYCPA = Lycopersicon parviflorum
- LYCPN = Lycopersicon pennellii
- LYCCI = Lycopersicon chilense
- LYCHI = Lycopersicon hirsutum
- LYCGL = Lycopersicon glandulosum
- LYCCH = Lycopersicon cheesmanii
- ORIGIN = Country of origin of tomato varieties (see following page for full forms).
- ACCNUM = Accession numbers of tomato varieties according to the Regional Plant Introduction Station, Iowa State University, Ames, Iowa or University of California, Davis, California.
- NUM = Number of treatment replications.
- UNTRTED = Fresh weights of untreated plants in grams.
- TRTED = Fresh weights of glyphosate treated plants.
- DIFF = Difference between fresh weights of untreated and treated plants expressed in percentage for all tomato varieties.
- SIGNIF = Significance levels based on t-test.
- All varieties with no asterisk showed a significant difference between untreated and treated plants at $P > 0.5$.
 - All varieties with one asterisk (*) showed no significant difference between untreated and treated plants at $P \geq 0.5$ and $P \leq 0.08$.
 - All varieties with two asterisks (**) showed no significant difference between untreated and treated plants at $P \geq 0.08$ and $P < 0.95$.
 - All varieties with three asterisks (***) showed no significant difference between untreated and treated plants at $P \geq 0.95$.
- All varieties with fresh weights of treated plants at least 10% higher than the untreated plants were also included in this group.

TABLE 2. REEVALUATION OF SOME TOMATO VARIETIES FOR GLYPHOSATE TOLERANCE IN THE GREENHOUSE.

LABEL	SCINAME	ORIGIN	ACCNUM	NUM	UNTRTED	TRTED	DIFF	SIGNIF
(PLANTED JUNE 4, 1984)								
126	LYCES	CHIN	92861	10	8.34	5.74	32.11	
129	LYCES	CHIN	92864	9	10.52	9.52	8.47	
132	LYCES	CHIN	93302	10	6.12	3.83	36.69	
551	LYCPE	PERU	128663	10	6.83	5.54	17.73	
561	LYCPE	PERU	129146	8	3.75	2.56	22.12	
562	LYCPE	ECUD	129149	10	2.17	0.87	21.54	*
563	LYCPE	ECUD	129152	10	4.22	2.50	21.75	
568	LYCES	BALU	135909	10	10.66	9.49	9.80	
570	LYCPE	ECUD	143679	10	7.58	3.40	57.59	
572	ES*PI	GERM	180725	10	9.11	4.27	51.86	
573	LYCPE	PERU	212407	10	5.43	1.91	60.42	
576	LYCPE	PERU	246585	10	5.96	3.57	40.64	
578	LYCPE	PERU	251302	4	1.02	0.32	63.84	
617	LYCPI	USA	375937	9	9.04	7.67	14.54	
620	LYCPI	PERU	379018	10	4.16	1.60	63.19	
647	LYCPI	ECUD	390519	10	5.77	4.33	24.08	
654	LYCPE	PERU	390664	9	4.64	3.24	29.73	
656	LYCPE	PERU	390679	10	2.84	1.02	61.10	
845	LYCES	VE NE	119215	10	9.39	4.83	47.79	
846	LYCES	VE NE	119446	10	9.62	3.19	66.44	
847	LYCES	ARGE	119776	10	9.75	3.79	59.80	
848	LYCES	ARGE	119777	10	9.45	5.11	49.63	
849	LYCES	ARGE	119778	10	10.05	4.28	58.63	
850	LYCES	TURK	120253	10	10.75	9.91	6.46	
851	LYCES	TURK	120254	9	9.26	4.07	59.67	
852	LYCES	TURK	120256	10	8.84	4.27	53.25	
854	LYCES	TURK	120258	10	9.05	3.28	64.25	
855	LYCES	TURK	120259	10	11.25	11.87	-6.51	
908	LYCES	PANA	126407	10	11.43	10.06	11.09	
909	LYCES	PANA	126408	10	10.92	10.47	3.16	*
910	LYCES	PERU	126409	10	7.40	2.30	67.08	
911	LYCES	PERU	126410	10	6.43	1.56	76.64	
912	LYCES	PERU	126411	9	5.52	1.28	77.28	
913	LYCES	PERU	126412	9	7.49	2.82	59.79	
914	LYCES	PERU	126413	10	8.46	8.98	-6.56	
915	LYCES	PERU	126414	10	9.51	9.77	-4.38	
916	LYCES	PERU	126415	10	9.36	4.66	49.12	
919	LYCES	PERU	126418	10	7.72	1.68	78.61	
920	LYCES	PERU	126419	10	8.59	2.95	64.45	
924	LYCES	PERU	126423	10	9.18	3.66	60.43	
928	LYCES	PERU	126427	10	8.46	2.51	69.55	

REEVALUATION OF SOME TOMATO VARIETIES FOR GLYPHOSATE TOLERANCE

LABEL	SCINAME	ORIGIN	ACCNUM	NUM	UNTRTED	TRTED	DIFF	SIGNIF
936	LYCGL	PERU	126440	10	7.08	5.14	26.88	
937	LYCPE	PERU	126441	10	4.19	1.45	64.93	
938	LYCGL	PERU	126443	10	7.27	5.67	21.66	
939	LYCGL	PERU	126444	10	3.73	1.06	71.20	
961	LYCES	PERU	126921	10	8.90	4.61	47.70	
964	LYCPI	PERU	126924	10	9.01	8.98	0.26	***
965	LYCPI	PERU	126925	10	8.88	7.70	12.02	
966	LYCPE	PERU	126926	10	6.91	5.20	23.65	
967	LYCPI	PERU	126927	10	8.91	9.50	-8.10	
968	LYCPE	PERU	126928	7	6.70	5.24	19.00	
973	LYCPI	PERU	126933	9	6.30	2.64	58.60	
974	LYCPI	PERU	126934	10	5.69	1.18	79.33	
1144	LYCES	ARGE	194561	10	7.75	2.39	69.02	
1149	LYCES	BRAZ	196481	10	9.31	9.61	-1.87	**
1156	LYCGL	PERU	199380	10	4.52	1.62	63.39	

Application of glyphosate at a later growth stage has less effect on the growth of tomato plants than application at an early growth stage of plants. Although the tomato varieties were grown in the greenhouse for both evaluations, the environmental conditions, such as hot sunny days at the time of treatment and immediately after can also affect the tolerance of tomato plants to glyphosate. The fact that tomato plants were grown during June (1984) when the days were hot and plants were growing vigorously could have reduced their tolerance to the herbicide in the repeat experiment.

It should be noted that the 56 tomato varieties evaluated in this experiment were selected from 1022 tomato varieties in the main screening program up to the end of 1983. There are many more tomato varieties that can be selected for reevaluation from the 500 more tomato varieties that have been screened since then.

Evaluation of Some Tomato Varieties for Glyphosate Tolerance in the Field (US)

Out of the 56 tomato varieties selected for reevaluation for glyphosate tolerance in the greenhouse, 30 varieties were tested for their tolerance to the herbicide in the field. All the 30 varieties were transplanted in the field during the summer of 1984.

As a result of glyphosate application, all tomato varieties showed the typical injury symptoms in the meristematic regions of the shoot within one week of treatment. Glyphosate affected the growth of tomato plants considerably as indicated by mean difference in vigor of untreated and treated plants. None of the tomato varieties showed vigor of treated plants greater than 75% of that of untreated plants towards the end of the season. Although some of the tomato varieties were able to overcome the initial inhibition of growth by glyphosate, all tomato varieties developed thin, spike-like leaves in the meristematic regions.

The effect of glyphosate on the height of tomato plants was much less than the effect on plant vigor or shoot diameter (Table 3). Out of 30 varieties tested, 16 showed shoot heights of treated plants to be greater than 30% of those of untreated plants. Five of these varieties showed no significant difference between the heights of untreated and treated plants at a probability level of 50% or greater and two of the varieties showed no significant difference between the heights of untreated and treated plants at a probability level of 95% or greater. This may be due to the fact that some tomato varieties were able to overcome the initial inhibition of growth of plants by glyphosate and developed long, spike-like leaves in the meristematic regions.

The effect of glyphosate on shoot diameter was significant on all except two tomato varieties. Shoot diameters of the treated plants of the two varieties labeled 928 and 939 were not significantly different from those of untreated plants at probability levels of 95% and 50% or greater, respectively. Shoot diameters of these two varieties appeared to compensate for reduction in heights of the plants due to glyphosate application (Table 3).

The fresh weight of shoots of tomato plants was significantly affected by glyphosate in all but three varieties. Two of the varieties labeled 562 and 1156 had shoot fresh weights of treated plants greater than those of untreated plants. These two varieties also showed considerable increase in shoot height of treated plants. Hence, it appears that the increase in shoot weight of treated plants of the two varieties may be due to increased size of plants (Table 4).

The effect of glyphosate application was most drastic on fruit production of tomato plants. The herbicide inhibited fruit development in

TABLE 3. EVALUATION OF SOME TOMATO VARIETIES FOR GLYPHOSATE TOLERANCE IN THE FIELD.

LABEL	NUM	UNTHT	THT	UNT DIA	TDIA	UNTVIG	TVIG	DIFHT	DIFDIA	DIFVIG
126	5	80.80	61.00	182.00	64.00	97.40	37.00	24.84	64.96	60.40
132	5	73.00	55.40	184.00	65.00	98.40	36.00	23.55	64.54	62.40
561	5	42.40	36.00	220.00	163.00	96.60	72.00	7.04*	25.91	24.60
562	4	36.00	43.75	153.75	83.75	93.50	62.50	-21.63**	43.18	31.00
570	5	81.20	60.00	168.00	74.00	99.00	42.00	25.21	55.22	57.00
572	4	70.00	63.75	192.50	96.25	95.25	51.25	8.42	49.53	44.00
845	5	73.00	63.00	200.00	77.00	99.00	47.00	13.65	61.22	52.00
846	5	81.40	61.00	157.00	70.00	98.60	43.00	24.17	55.43	55.60
847	5	66.40	53.00	166.00	75.00	97.60	42.00	17.63	53.64	55.60
848	4	80.00	56.25	166.25	68.75	95.75	28.75	28.28	59.40	67.00
849	5	70.00	50.00	155.00	65.00	99.00	34.00	28.32	58.07	65.00
852	5	75.40	67.00	187.00	78.00	98.20	47.00	10.16	58.37	51.20
854	5	65.00	57.00	181.00	64.00	98.40	41.00	10.41	64.72	57.40
857	3	76.67	53.33	203.33	80.00	95.00	38.33	30.28	60.52	56.67
910	5	89.00	78.00	204.00	90.00	97.60	47.00	11.07	55.93	50.60
911	5	84.00	65.00	159.00	85.00	97.60	43.00	22.27	42.22	54.60
912	5	78.40	58.40	171.00	81.00	90.20	50.00	24.38	41.54	40.20
913	5	66.00	62.60	187.00	105.00	94.00	49.00	3.58*	41.38	45.00
916	5	73.80	61.00	164.00	99.00	95.80	53.00	16.01	38.78	42.80
919	5	68.00	55.00	173.00	100.00	99.20	59.00	18.74	41.93	40.20
920	5	84.00	70.00	206.00	81.00	93.00	38.00	16.74	60.80	55.00
924	5	66.60	67.00	199.00	106.00	97.60	51.00	-4.63*	46.71	46.60
928	5	73.00	55.00	129.00	67.00	97.80	39.00	24.44	-5.05**	58.80
937	5	47.60	37.00	191.00	107.00	99.00	51.00	22.11	40.74	48.00
939	5	54.60	40.60	153.00	132.00	97.20	63.00	25.47	10.27*	34.20
961	5	77.40	59.00	213.00	101.00	95.40	51.00	21.11	52.35	44.40
973	5	65.00	53.00	223.00	86.00	91.80	46.00	16.29	61.39	45.80
974	5	53.00	39.00	252.00	117.00	93.00	63.00	18.78	53.15	30.00
1144	5	75.00	48.60	146.00	58.00	96.80	30.00	35.43	57.02	66.80
1156	5	24.40	30.40	225.00	142.00	93.20	68.60	-32.91**	37.87	24.60

LABEL = Code numbers of tomato varieties given at VPI&SU, Blacksburg, Virginia.

NUM = Number of treatment replications.

UNTHT = Height of untreated plants in cm.

THT = Height of glyphosate treated plants in cm.

UNT DIA = Shoot diameter of untreated plants in cm.

TDIA = Shoot diameter of glyphosate treated plants in cm.

UNTVIG = Percent vigor of untreated plants.

TVIG = Percent vigor of glyphosate treated plants.

DIFHT = Percent difference in height of untreated and treated plants.

DIFDIA = Percent difference in shoot diameter of untreated and treated plants.

DIFVIG = Percent difference in the vigor of untreated and treated plants.

*following DIFHT or DIFDIA refers to no significant difference at $P > 0.5$ and $P < 0.8$. **following DIFDIA refers to no significant difference at $P \geq 0.8$ and $P < 0.95$.

all varieties except one (Table 4). In some varieties, no fruits were produced as a result of glyphosate treatment. It is interesting to note, however, that the tomato variety labeled 1156, which had no significant difference between fruit weights of untreated and treated plants at a probability level of 50% or greater, also showed increased height and fresh weights of shoots of treated plants as compared to many other tomato varieties. Thus, it appears that tomato variety labeled 1156 has some potential for tolerance to glyphosate application in the field and should be included in future evaluations for glyphosate tolerance.

Effect of Glyphosate on Young Transplanted Tomato Plants in the Field (US)

In addition to the 30 tomato varieties evaluated in the field, nine tomato varieties, including PUZ II, a variety reported to be partially resistant to broomrape, were evaluated in the field for glyphosate tolerance. Twenty plants or less of each of the nine varieties were transplanted in the field in two rows of ten plants each 21 days after planting in the greenhouse. There were five replications in the experiment. The plants were treated 14 days after transplanting when they were in the 8 to 10 leaf stage and observations were recorded 21 days after treatment.

All tomato varieties showed typical symptoms of glyphosate injury in the shoot terminal meristem. The injury symptoms included chlorosis and bleaching of the plant tissue in the terminal shoot of leaves. The diameter of the area showing the injury ranged from 4.74 cm to 8.98 cm (Table 5); however, due to variability in the data there were no significant differences between the extent of injury to the tomato varieties.

TABLE 4. EVALUATION OF SOME TOMATO VARIETIES FOR GLYPHOSATE TOLERANCE IN THE FIELD.

LABEL	NUM	UNTSWT	TSWT	UNTFWT	TFWT	DIFSWT	DIFFWT
126	3	22.40	5.70	15.83	2.58	72.22	82.14
132	3	23.30	4.37	10.07	0.37	79.93	96.08
561	3	6.47	4.70	1.80	1.15	27.52	28.55
562	3	8.17	5.23	2.37	0.43	-49.88*	67.93
570	3	23.43	6.00	15.03	1.13	73.36	91.54
572	3	17.33	6.57	13.83	3.07	62.06	78.49
845	3	25.97	7.53	12.47	1.03	68.59	91.46
846	3	21.83	4.63	14.63	0.57	78.98	96.45
847	3	21.87	4.27	14.90	0.53	81.19	96.82
848	3	19.17	3.13	13.03	0.10	81.21	99.21
849	3	15.73	3.40	12.53	0.63	77.97	95.81
852	3	13.43	5.80	7.73	1.47	55.01	79.73
854	3	12.60	4.57	8.60	1.38	63.19	85.01
857	3	19.40	5.90	14.80	2.03	69.47	85.92
910	3	23.27	6.20	12.67	0.77	72.65	95.63
911	3	13.57	4.30	15.40	1.75	47.16	86.94
912	3	26.57	8.20	16.00	1.13	67.99	92.78
913	3	17.77	8.60	11.00	2.73	54.38	75.74
916	3	13.60	6.07	7.50	1.20	55.08	82.52
919	3	13.43	7.47	8.17	1.47	44.54	81.29
920	3	18.73	2.53	13.47	0.73	86.47	94.49
924	3	18.97	8.53	13.27	2.03	40.54	75.63
928	3	17.70	5.77	10.80	0.43	65.38	95.57
937	3	6.20	4.00	2.23	0.97	33.41	52.11
939	3	5.93	4.00	1.67	0.90	12.03**	34.30
961	3	22.33	6.80	17.00	1.90	64.86	88.05
973	3	18.20	5.37	8.87	3.53	64.90	53.31
974	3	13.23	3.97	5.90	0.20	46.91	95.84
1144	3	19.40	3.03	16.40	1.37	84.60	92.02
1156	3	4.97	4.77	1.70	0.73	-40.06 *	14.91*

 LABEL = Code numbers of tomato varieties given at VPI&SU, Blacksburg, Virginia.

NUM = Number of treatment replications.

UNTSWT = Shoot fresh weight of untreated plants in grams.

TSWT = Shoot fresh weight of glyphosate treated plants in grams.

UNTFWT = Fruit weight of untreated tomato plants in grams.

TFWT = Fruit weight of glyphosate treated tomato plants in grams.

DIFSWT = Percent difference in shoot fresh weights of untreated and treated plants.

DIFFWT = Percent difference in fruit weight of untreated and treated tomato plants.

*following a DIFSWT or DIFWT refers to no significant difference at $P \geq 0.5$ and $P < 0.8$.

**following a DIFSWT refers to no significant difference at $P \geq 0.8$ and $P < 0.95$.

All plants, untreated and glyphosate treated, were affected by a light frost that occurred during the period of the experiment. As a result, vigor of glyphosate-treated plants was recorded relative to that of untreated plants. The vigor of all glyphosate-treated plants was reduced as compared to untreated plants (Table 5). No significant differences were observed, however, between the vigor of the tomato varieties.

The dry weights of shoots of all glyphosate-treated plants, except those of one variety labeled 129, were reduced as compared to those of untreated plants (Table 5). The variety labeled 129 showed tolerance to glyphosate, since there were no significant differences between the shoot dry weights of untreated and treated plants at a probability level between 80 and 95% ($P \geq 0.8$ and $P \leq 0.95$). It is possible, however, that injury due to frost could have exerted a stress on growth of tomato plants and affected their tolerance to glyphosate. It is suggested, therefore, that all the nine tomato varieties should be included in future experiments for glyphosate tolerance.

TABLE 5. EFFECT OF GLYPHOSATE ON YOUNG TRANSPLANTED TOMATO PLANTS IN THE FIELD.

LABEL	NUM	INJDIA	RELVIG	UNTDWT	TDWT	DIFDWT
129	3.	8.37	3.42	3.78	2.17	11.49**
551	5	6.58	4.55	6.42	3.39	45.36
568	5	7.36	4.10	5.70	3.15	43.65
850	5	8.46	5.10	7.91	3.63	47.14
855	5	8.98	5.20	5.44	3.79	28.19
908	5	7.78	5.15	5.84	3.21	44.57
914	5	7.28	4.05	4.66	2.80	36.18
915	5	8.26	4.30	4.70	2.69	43.40
PUZ11	5	4.74	4.20	1.62	1.06	33.01

 LABEL = Code number of tomato varieties given at VPI&SU, Blacksburg, Virginia.

NUM = Number of treatment replications. Each replication consists of 10 or fewer plants.

INJDIA = Diameter of the area of shoot meristem showing injury symptoms of glyphosate in cm.

RELVIG = Relative vigor of glyphosate treated plants as compared to vigor of untreated plants on a scale of 0 to 10. A score of 0 meaning no injury and a score of 10 meaning complete kill.

UNTDWT = Dry weight of untreated plants in grams.

TDWT = Dry weight of treated plants in grams.

DIFDWT = Percent difference between the dry weights of untreated and treated plants.

** following a DIFDWT refers to no significant difference at $P \geq 0.8$ and $P < 0.95$.

Evaluation of Some Tomato Varieties for Glyphosate Tolerance in The Field
(Israel)

The experiment was originally planned with three replications. However, broomrape did not appear in the field although it was infested according to the experiment station personal. Therefore, there was no difference between the fumigated and non-fumigated plots and the experiment was treated as having six replications. As mentioned in the section of materials and methods we did not proceed with harvesting yield. Therefore, we emphasize the value of the visual evaluation which took into account growth retardation, yellowing of the leaves and particularly, spindliness. Spindliness is a typical response of tomato plants as well as other plants, to low rates of glyphosate. Fewer and smaller spike-like leaves are formed. The larger leaves are often rolled. Flowering is a very sensitive process to glyphosate. Less flowers are formed or drop leaving bare flower stalks. The figures are presented in Table 6 a.

The varieties labelled 551 and 961 demonstrated the most consistent tolerance to glyphosate. However, it seems that the damage inflicted by 50 gr/ha of glyphosate is too high even for those two varieties and lower rates will have to be studied with regards to crop damage and broomrape control.

Table 6a. Effect of two rates of glyphosate on various tomato varieties as determined by visual evaluation and measured by plant height.

Plant height				Visual evaluation (1-5)			
Glyphosate		Glyphosate		Glyphosate		Glyphosate	
50 gr/ha		100 gr/ha		50 gr/ha		100 gr/ha	
% of		% of		% of		% of	
label	check	label	check	label	check	label	check
551	98	551	86	961	3.9	961	2.6
961	90	961	72	974	3.4	551	2.5
1156	83	974	71	551	3.4	572	2.1
939	81	129	69	939	3.4	129	2.0
126	81	126	69	572	3.2	126	1.9
620	79	1156	68	911	3.1	974	1.9
129	78	620	68	129	3.1	132	1.8
851	75	5-5	68	126	3.0	5-5	1.8
974	74	939	64	1156	2.8	620	1.7
568	73	568	63	620	2.7	851	1.7
911	73	572	61	132	2.7	568	1.7
S-5	71	848	61	S-5	2.6	939	1.7
572	71	851	60	851	2.6	848	1.6
848	69	132	57	848	2.5	1156	1.6
132	67	911	54	568	2.4	578	1.4
578	64	578	46	578	2.3	911	1.2

1. Values connected by a line do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

2. Visual evaluation: 1- Severe damage 5 - No visible damage.

Table 6b: Accession numbers, scientific names and origin of the labels
(varieties) that are listed in part a.

Label	No.	Name	Origin
126	92861	LYCES	CHINA
129	92864	LYCES	CHINA
132	99302	LYCES	CHINA
551	128663	LYCPE	PERU
568	135609	LYCES	BALUCHISTAN
572	180725	ESXPI	GERMANY
578	251302	LYCPE	PERU
620	379018	LYCPE	PERU
848	119777	LYCES	ARGENTINA
851	120254	LYCES	TURKEY
911	126410	LYCES	PERU
939	126444	LYCGL	PERU
961	126921	LYCES	PERU
974	126934	LYCPI	PERU
1156	199380	LYCGL	PERU
S-5	check	LYCES	ISRAEL

Legend - as for Table 2.

Screening Tomato Varieties for Resistance to Egyptian Broomrape (Israel)

Preliminary experiments

The mixture of claysoil, sand and tuft at a ratio of 1:1:1 (by volum) gave very good and consistant infection on the roots of tomato plants. This mixture also released itself nicely from the roots and the young broomrape infection were clearly visible without having to wash the roots, which would have been laborious and time consuming. There was no advantage in loading the potting mixture with more than 50 mg of dry egyptian broomrape seeds per pot. 25 mg of seeds per pot gave less consistant infection than 50 mg.

Screening Tomato Varieties for Tolerance to Egyptian Broomrape (Israel)

Main Experiments

First year - Spring 1982

530 varieties were included in the experiment. 38 varieties (7%) were graded "infection level 1" or less (low enough to counted) in two or three pots out of the six provided growth of the tomato plants, normal (graded 4 or above on a 1 to 8 scale). Non of the varieties showed consistant resistance.

Second year - Spring 1983

Shortly after planting the second experiment we discovered that the soil we received to prepare the potting mixture contained Atrazing residues. The first experiment (planted May 5) mostly survived the residues. Broomrape evaluation was done one June 23, namely 49 days after transplanting. 168 varieties were found with heavy broomrape infection. All

14 varieties that had lower infection. The first year and were retested the second year were also found with heavy broomrape infection.

Information about 49 varieties is missing due to missing plants as a result of poor germination or early collapse because of the Atrazin residues. The second planting was even less fortunate. Temperatures were higher and the plants were totally stunted and eventually the experiment was discarded.

Third experiment spring 1984.

663 out of the 703 varieties received from the Davis-California collection were screened. 40 varieties had non or poor germination. Ten varieties showed no infection or only very few. Those varieties were very few. Those varieties were:

Screening number	Species	Country of origin	Identification No.
1266	L. esc. V.ceras	Ecuador	LA 292 76 L 1206 op
1204	L. pimpinellifolium	Peru	LA 1585 75 L 214L mass 0
1429	L. "	"	LA 1469 74 L 320-2 0
1529	L. "	"	LA 2401 82 L 2783 mass op
1530	L. "	"	LA 2391 82 L 2781 mass op
1653	L. "	"	LA 1614 79 L 3686 mass op
1701	L. "	"	LA 2183 81 L 680 mass op
1716	L. Hirsutum	"	LA 1353 74 L 2460 1-6 sib
1726	L. pimpinellifolium	"	LA 1645 75 L 2303 0
1812	L. esc. V. ceras	Ecuador	LA 2137 81 L 652 mass op
1814	L. " "	Peru	LA 2313 81 L 795 mass op

Those plants were replanted and seeds produces.

Screening Tomato varieties for Resistance to Egyptian Broomrape

Confirmation Experiment (Israel)

The seed produced in the summer of 1984 were used for the confirmation experiment. All the varieties were infected with Egyptian broomrape in this experiment in both lower and higher rates of infestation.

Total of 1361 entries were screened for resistance to Egyptian broomrape, covering a wide range of the existing genetic sources of the tomato and related wild species. Representatives of following species and subspecies were present in the screening program:

- L. esculentum
- L. esculentum, var. cerasiforme
- L. pimpinellifolium
- L. glandulosum
- L. peruvianum
- L. peruvianum, var. humifusum
- L. hirsutum f. glabratum
- L. cheesmani
- L. pennellii
- L. chilense
- L. parviflorum
- S. richii
- S. Lycopersicoides

No resistance to Egyptian broomrape was found. The method was such that partial resistance could not have been identified, and most likely would have been of little practical value. The chances to find resistance in the future in those species which are self polination are probably smaller because of their greater uniformity.

Effect of Soil Mixtures on Broomrape Infestation on Tomatoes in the Greenhouse (US)

Broomrape seeds do not germinate readily, but require proper soil type, moisture and temperature to germinate and infect host plants. To investigate the effect of soil type on broomrape infestation on tomatoes, three types of soil mixtures were tested in the greenhouse. Tomato varieties used in this experiment were 'Rutgers', a commercial variety, and 'PUZ II', a variety reported to be partially resistant to O. aegyptiaca (Avdeev and Shcherbinin, 1978).

In general, broomrape infestation occurred much more and sooner in soil mixtures A and B than in the soil mixture C (Table 7). Broomrape shoots were visible in about six weeks in almost all pots containing soil mixtures A or B. Broomrape shoots emerged in about seven weeks in only two of the 24 pots containing soil mix C. The growth of tomato plants in the three types of soil mixtures differed considerably. Tomato plants in soil mix C grew much more vigorously than tomato plants in soil mix A or B as evidenced by their shoot height and shoot and root fresh weights (Table 7).

PUZ II tomato plants appeared to grow more vigorously than Rutgers tomato plants. This more vigorous growth of tomato plants in soil mix C may be the reason for their poor susceptibility to broomrape. It has been reported that plants growing in marginal soils with low nutrient levels are more susceptible to infection by broomrape than plants growing in more fertile soils (Abu-Irmaileh, 1979). Also, the physical characteristics of the soils, such as the water holding capacity, may influence broomrape infection on host plants. Soil mix C with a higher vermiculite content had a greater water holding capacity than soil mix A or B.

TABLE 7. EFFECT OF SOIL MIXTURES ON BROOMRAPE INFESTATION ON TOMATOES IN THE GREENHOUSE.

Treatment*	Tomato**			Broomrape		
	Shoot ht. (cm)	Shoot f.w. (g)	Root f.w. (g)	No. of inf.	Shoot length (cm)	Fresh weight (g)
<u>Rutgers</u>						
Soil mix A	10.88	4.68	2.48	2.25	5.52	1.60
Soil mix B	13.52	8.80	7.39	4.38	5.77	2.73
Soil mix C	67.25	81.10	20.87	0.25	0.54	1.40
LSD _{.05}	3.86	11.27	5.52	1.70	3.89	3.48
<u>PUZ II</u>						
Soil mix A	26.25	13.38	8.23	4.50	3.65	2.70
Soil mix B	26.17	3.38	1.96	5.78	3.20	2.61
Soil mix C	92.50	89.38	--	0.25	1.19	1.55
LSD _{.05}	6.61	4.81	7.71	1.87	1.74	4.34

* Soil mix A = clay loam (33.3%), sand (33.3%), and weblite (33.3%).
 Soil mix B = clay loam (45%), sand (45%), and peat moss (10%).
 Soil mix C = vermiculite (40%), weblite (40%), and peat moss (20%).

LSD_{.05} = least significant difference values at 5% level of significance.

** f.w. = fresh weight. In case of root fresh weight of PUZ II tomatoes, root fresh weights from soil mix C were not available.

Both Rutgers and PUZ II varieties appeared equally susceptible to broomrape (Table 7). Although PUZ II tomato plants appeared to grow slightly more vigorously than Rutgers tomato plants, the number of broomrape infections were slightly higher in the former than the latter variety, especially in soil mixtures A and B. There was no apparent difference in the time of emergence of broomrape on the two varieties. The reason for high susceptibility of PUZ II tomato plants to broomrape in our experiments is not known.

Translocation of ^{14}C -Glyphosate in Tomato and Broomrape (US)

Broomrape being an obligate parasite sustains its growth by drawing nutrients and water from the host plant. In order to investigate if the parasite would also withdraw a readily translocating herbicide, such as glyphosate, from the host ^{14}C -glyphosate was applied to the leaves of tomato plants. Observations seven days after glyphosate application indicated that there was no visible injury to the host or the parasite from the herbicide. The distribution of radioactivity in tomato plants infected with broomrape three and seven days after treatment are illustrated in Figures 3 and 4 for Rutgers and Figures 5 and 6 for PUZ II tomato variety.

The radioactivity appeared to move quite readily in both Rutgers and PUZ II tomato plants and into the attached broomrape plants. The translocation of radioactivity to the treated leaf margins appeared to occur via the apoplast while translocation of radioactivity to broomrape possibly occurred via the symplast. There appeared to be a greater accumulation of radioactivity in the growing points of broomrape shoots than in the growing points of the shoot and roots of the host plant. This may be due to the fact that broomrape acts as a much stronger sink for nutrients and water than the growing points of the host plant. A greater translocation and accumulation of glyphosate in broomrape shoots than in

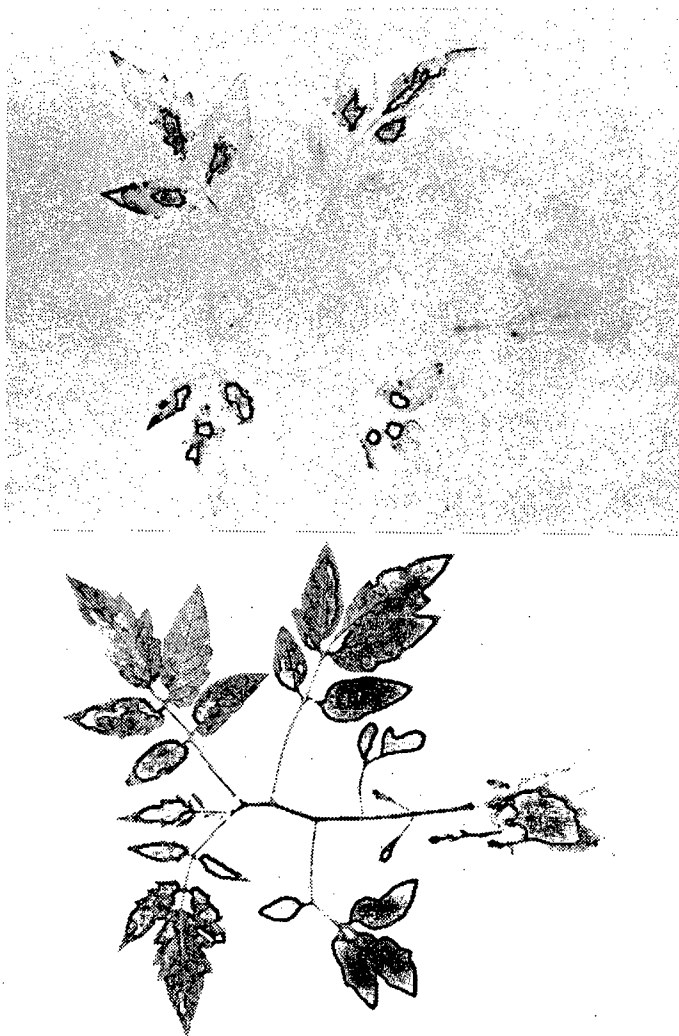


Figure 3. Dry mount and autoradiogram of tomato (cv. Rutgers) plant 3 days after treatment with ^{14}C -glyphosate.

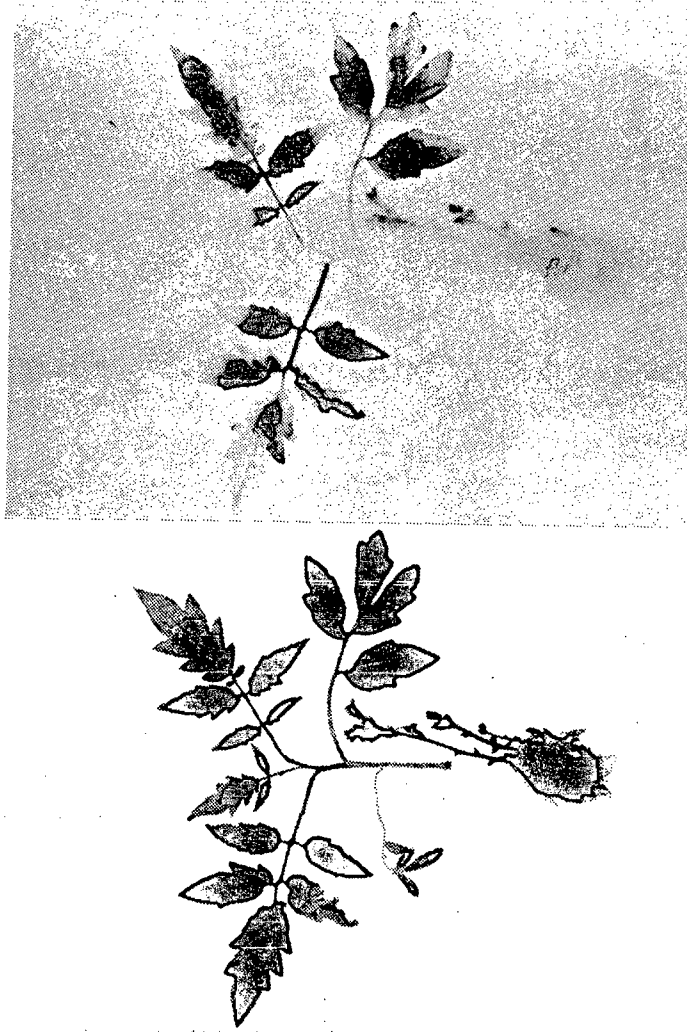


Figure 4. Dry mount and autoradiogram of tomato (cv. Rutgers) plant 7 days after treatment with ^{14}C -glyphosate.

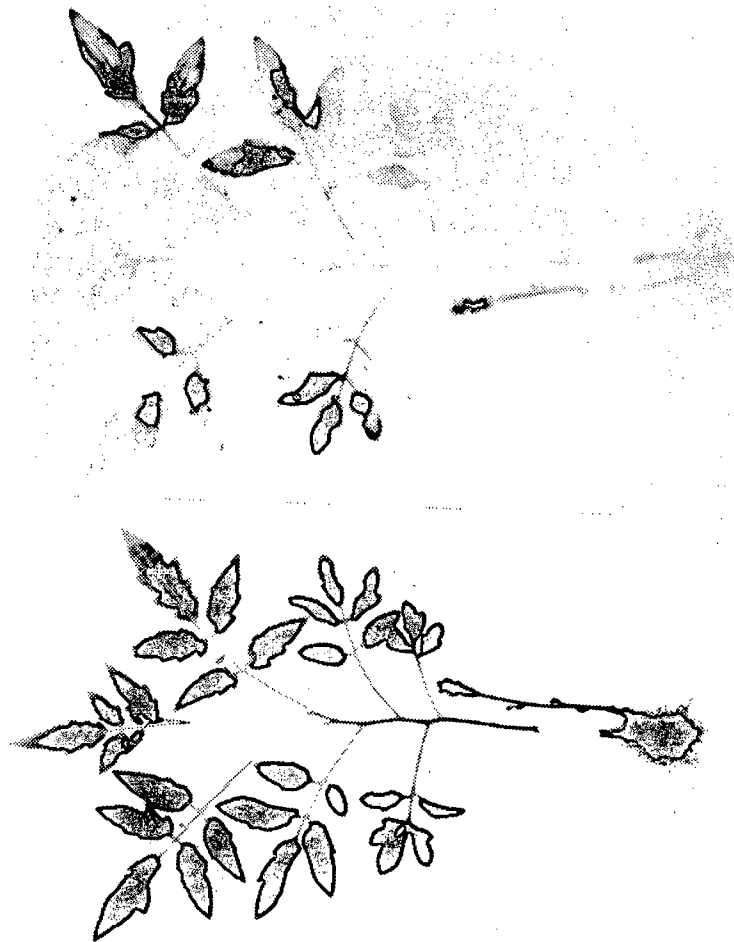


Figure 5. Dry mount and autoradiogram of tomato (cv. PUZ II) plant 3 days after treatment with ^{14}C -glyphosate.

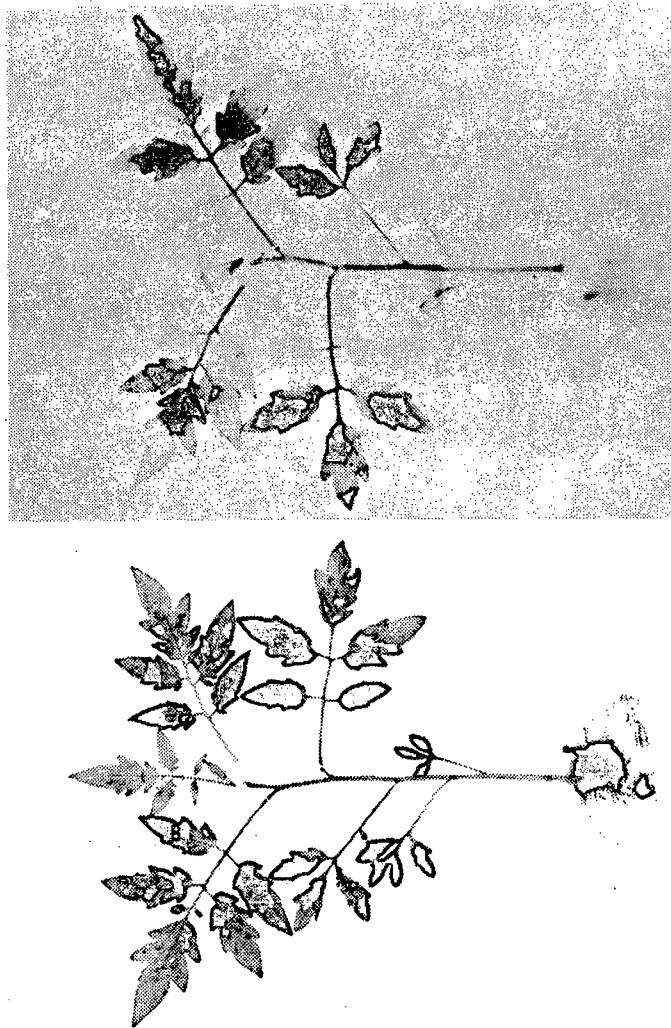


Figure 6. Dry mount and autoradiogram of tomato (cv. PUZ II) plant 7 days after treatment with ^{14}C -glyphosate.

the host tissues in highly desirable in order to achieve selective control of the parasite in some crops by glyphosate.

The nature of radioactivity translocated to broomrape from host leaves could not be determined by the time of writing this report. It is highly possible, however, that the radioactivity present in broomrape was associated with intact glyphosate. It has been observed that glyphosate is metabolically stable in many plant species (Gottrup *et al.*, 1976; Schultz and Burnside, 1980). It has also been reported that glyphosate is very effective against broomrape when applied to crop plants such as broad beans at very low rates (60 to 120 g/ha). It suppresses and/or controls the attached parasite without adversely affecting the more tolerant host plants (Kasasian, 1973; Jacobsohn and Kelman, 1980; Schmitt *et al.*, 1979; Schluter and Aber, 1979). Our research appears to confirm the report that glyphosate translocates from host leaves to broomrape shoots.

Infecting Tomato Plants in a Soilless System (Israel)

The value of the method (illustrated in figures 7-10) is that it provides a useful research tool for studying the very initial stages of the root infection by the broomrape (or other phanerogamic root parasites). The advantage of the method is that it allows continuous observation and simple access to the infected roots by removing the glass and returning it, if desired, without disturbing the root system.



Figure 7. Infecting tomato plants with broomrape soilless system. The glass unit in a glass beaker.

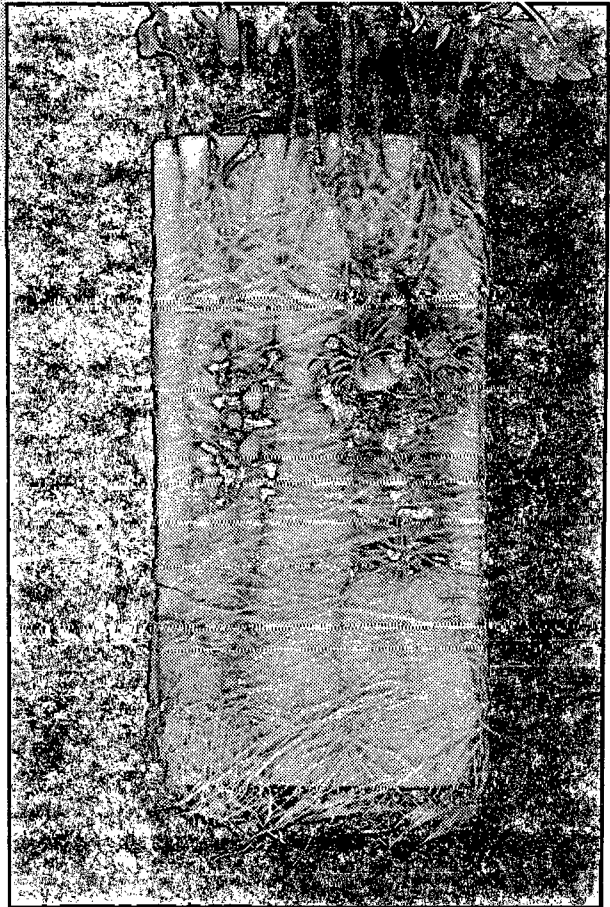


Figure 8. Nodding (left) and Egyptian (right) broomrapes infecting tomato plants.



Figure 9. Flowering Egyptian broomrape infecting tomato plants in a soilless system. Root kept in darkness.

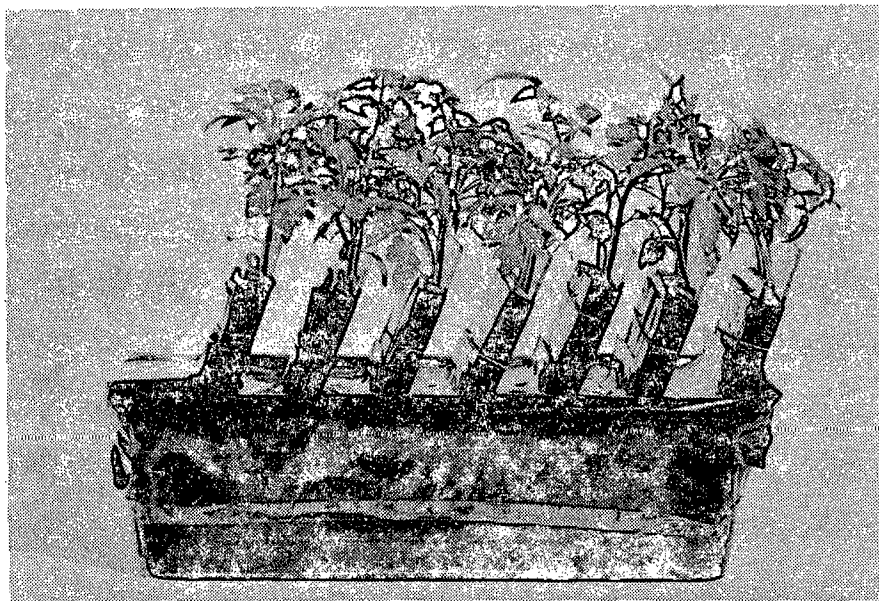


Figure 10. An arrangement of seven units in a container. Roots kept in darkness.

a. Replacing the whatman No. 1 filter paper by a synthetic cloth.

The units with the synthetic cloth provided adequate condition for ample infections and there were no visible contaminations during the experiment. Contaminations started to appear on the filter paper after 13 days. There was no significant difference in the number of infections.

b. Pre-conditioning the seeds and stimulating them to germinate.

Results of the experiment are presented in table.

Table 8. Effect of treating Egypti broomrape seeds with a synthetic germination stimulant prior to inoculation on the number of infections.

Infection	Germination stimulant		Difference
	+	-	
Small 1)	12	20	8 MS ⁴⁾
Medium 2)	35	38	3 NS
Large 3)	25	17	8 *
Total	72	75	3 NS

1. Small infection - Round haustorium, 2-3 mm in size.

2. Medium infection - Haustorium developed "roots" No apex visible.

3. Large infection - Apex clearly visible and starts to elongate.

4. NS = Not significant * - significant $P < 0.05$

There was no difference in the total number of infections resulting from stimulated and non-stimulated seeds. However, there were significantly more large infections in the units treated with stimulated seeds, suggesting that more rapid germination of the stimulated seed enabled earlier infection they had longer time to grow until termination of the experiment.

Improving Broomrape Seed Germination in the Laboratory

Seed disinfection

Although the usual procedure of disinfecting broomrape seeds with sodium hypochlorite was generally satisfactory, we thought it important to have other methods also available. It is also claimed that calcium and sodium hypochlorites have positive effect on broomrape seed germination. However, considerably longer exposure periods have been studied. The results of our experiments testing the effect of calcium and sodium hypochlorites and ethanol are presented in table 9.

Table 9. Effect of various disinfectants on broomrape seed germination.

Concentration (a.i)	Ethanol						Sodium hypochlorite						Calcium hypochlorite					
	50%		70%		90%		1%		3%		5%		1%		2%		3%	
Exposure Time (min)	1	5	1	5	1	5	1	5	1	5	1	5	1	5	1	5	1	5
----- % germination -----																		
<i>Orobancha aegyptiaca</i>	85	84	86	87	83	73	94	92	82	93	92	89	88	93	91	95	80	83
<i>Orobancha crenata</i>	11	12	12	11	12	17							54	49	47	47	37	51

Sodium hypochlorite was satisfactory in all treatments, and our usual procedure of 2% active chlorine for 5 minutes falls within the range of concentration and exposure time. Calcium hypochlorite did not reduce percent of germination but disinfection was not complete. Ethanol effectively disinfected the seeds. However, the ethanol treatment caused some reduction in germination of egyptian broomrape seeds compared to germination following hypochlorite treatment. The response of Egyptian broomrape is particularly mentioned because its germination is usually most stable and of high percentage. Much less reproducible is the germination of crenate broomrape.

The difficulties to obtain reproducible results in broomrape seed germination experiments are well known, It is our experience that most consistant germination is obtained with Egyptian (as mentioned) and Mutelli broomrape. Least consistence results are obtained in trying to germinate Crenate broomrape. Nodding broomrape is inbetween the two groups. The degree of reproducibility may depend also on the seed lot. Two different seed lots of the same species may respond very differently. Mallet (persoanl communication) speculateld that possibly the seeds have some kind of cycle of low germination periods. Such cycles were never proven.

Seed washing

As part of the efforts to overcome the difficulties just described, we studied the importance of the amount of water necessary to wash the seeds after surface disinfections. Results are presented in Table 10.

Table 10. Effect of amount of water used to wash seeds (200 mg) after surface disinfection with Sodium hypochlorite on Nodding broomrape seed germination.

Amount of water (cc)	% Germination	
	Exp. I	Exp. II
2 x 12.5	57 \pm 2.0	62 \pm 8.1
2 x 25	91 \pm 1.4	70 \pm 4.8
2 x 50	82 \pm 3.1	88 \pm 2.7
2 x 100	90 \pm 0.7	
4 x 50	88 \pm 3.5	82 \pm 1.2

In the first experiment only the first treatment resulted in a lower germination and in the second experiment the first two treatments resulted in lower germination. We suggest that these results are due the the detrimental effect of sodium hypochloride that was incompletely removed by the smaller amounts of water. It is also suggested that the amount of water used for washing the broomrape seeds is not a contributing factor to the variability of broomrape seed germination because generally at least two times fifty milliliters of water are used.

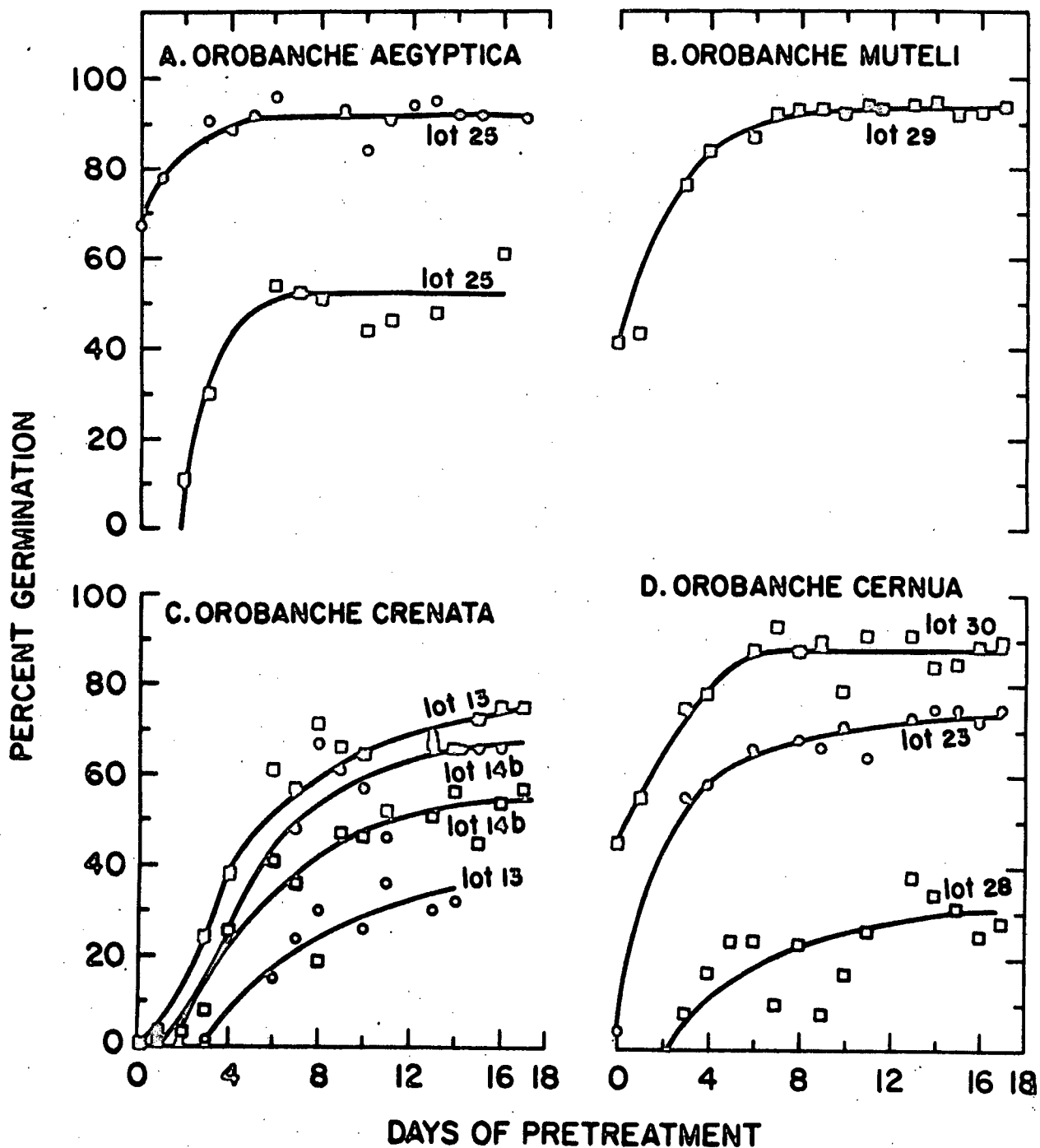
Pre-treatment period

Our standard broomrape seed germination test included a ten-days pretreatment period, namely keeping the seeds wet on fiber glass filter paper. In a series of experiments we tested whether or not the length of the pretreatment period can be changed. The results are presented in Figure 11. Each line presents a separate germination test. There are considerable differences in the germination from test to test. This variability is

widely stated throughout the literature and is indeed a problem. Egyptian (Fig. 11A) and Muteli (11B) broomrape are reaching maximum germination after six or seven days. Shortening the pretreatment period to that length is also appreciated because it is easier to plan the work on a weekly basis. The germination tests of these two species are mostly producing high germination counts (85-95%) and are relatively reproducible. Therefore, the low counts of one of the Egyptian broomrape tests presented in Figure 11A is an exception. Most unpredictable are the germination tests of Crenate broomrape (Fig. 11C). The highest and lowest lines represent two tests of the same seed lot (no. 13). The results also indicate that a 10 days pretreatment period of the seeds of this species is not sufficient to obtain maximum germination.

Considerable differences in germination are also obtained with Nodding broomrape (O. cernua) (Fig. 11D). In two of the experiments, a 10-days pretreatment period was not sufficient for maximum germination. Therefore, we are extending the pretreatment period for both Crenate and Nodding broomrape to 14 days.

Figure 11. EFFECT OF LENGTH OF PRETREATMENT PERIOD ON BROOMRAPE SEED GERMINATION



Temperature effect

Results are presented in Table 11.

Table 11. Temperature effect on percent germination of various broomrape species seeds.

Broomrape / Host	Temperatures (centigrate)					
	9	15	20	26	31	36
0. crenata / carrot	0	30 \pm 4.9	86 \pm 4.0	63 \pm 0.8	9 \pm 4.2	0
0. aegyptiaca/carrot	0	70 \pm 4.5	90 \pm 1.8	74 \pm 0.6	17 \pm 3.8	0
0. aegyptiaca/tomato	0	77 \pm 2.2	95 \pm 1.5	94 \pm 0.3	90 \pm 1.5	0
0. cernua / tomato	0	47 \pm 4.8	80 \pm 2.1	77 \pm 1.5	59 \pm 6.0	0
0. cernua / sunflower	0	33 \pm 2.9	93 \pm 0.5			
0. muteli/ potato	0	38 \pm 2.9	94 \pm 1.0	95 \pm 0.4	89 \pm 1.5	0

Highest germination occurred at 20°C for all species. Considerable differences in germination are observed in the extremes which possibly may indicate as to their seasonal activity.

The germination at 31°C of 0. aegyptiaca/carrot and 0. aegyptiaca/tomato was 17% and 90% respectively are of particular interest. Those results may support the hypothesis that those two populations of Egyptian broomrape represent two different physiological races.

From the laboratory point of view the results clearly indicate that 20°C is the optimal temperature for germination tests.

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F. DESCRIPTION OF COOPERATION

The two investigators cooperated at all levels of conducting the research as follows:

1. Jointly discussed and defined the research goals and objectives.
2. Mutual advise and consultation on the specific design of experiments.
3. Mutual assistance of in obtaining plant material. (Broomrape seeds from Israel and tomato varieties from Ames, Iowa and Davis, California - USA). Also, exchanged information and training on specific research techniques.
4. Research activities of the specific aspects of the same overall project were investigated in the different laboratoris. Decisions as to what experiments are to be conducted in Israel and/or USA as outlined in the proposal were rationally based on such factors as the interest and experience of the investigators, quarantive restriction and the availability of broomrape - infested crop land, specialized scientific equipment and facilities etc.
5. Mutual visits of the investigators at each of the institutes for evaluation of the research activities and results. Continuous exchange of information and results was carried out also by mail during the research.
6. The investigators jointly published already part of the research results and will continue to do so based on the level of contribution of the investigator and other personal under their supervision.

G. EVALUATION OF RESEARCH ACHIEVEMENT WITH RESPECT TO THE ORIGINAL RESEARCH PROPOSAL.

Primary objectives

1. Search for tomato varieties which have demonstrable and practical levels of tolerance to glyphosate.

Several tomato varieties have shown to demonstrate some tolerance to glyphosate. However, it is not yet determined whether or not those levels of tolerance are sufficient to allow the use of glyphosate as a selective herbicide for broomrape control in tomatoes.

The results are encouraging to continue further research with those varieties, particularly those belonging to the genus Lycopersicon various rates and time of application of the herbicide. The possibility of using various spray additives to try to reduce phytotoxicity should be experimented.

2. Search for tomato varieties which have demonstrable (and practical) levels of resistance to Egyptian broomrape.

No resistance to Egyptian broomrape was found within 1361 varieties that were screened. It is possible, although purely speculative, that the strain of the Egyptian broomrape used in the screening program is particularly virulent to tomatoes, and that other populations (strains) of Egyptian broomrape will be less virulent.

In fact, the variety PUZ II was reported to be partially resistant in the USSR but was susceptible in our experiments. For future work, and in a situation of limited resources, we would not recommend to try this avenue in searching for a solution to the broomrape problem in tomatoes. It might

we worthwhile to conduct a comparative study of the virulence of various populations of Egyptian broomrape.

Secondary objectives

3. To study Transport of Foliage Applied Glyphosate from the Host to the Parasite.

This study, employing ^{14}C -glyphosate radiotracer methodologies is an essential phase in the development of practical usage of glyphosate.

The results prove that radioactivity moves quite readily in the tomato plant and into the attached broomrape. A massive move of radioactivity to the broomrape growing points indicates that the broomrape acts as a powerful sink. Those findings are both of scientific value and practical value supporting the hypothesis that glyphosate might be suitable for selective control of broomrape in tomatoes.

4. To Develop a Rapid Method of Infecting Tomato Roots with Broomrape in vitro.

A method was developed to obtain broomrape infection behind glass in a soilless system. Infections of Egyptian broomrape can be observed after 2.5-3 weeks from initiation of the experiment. This rapid infection is achieved by using broomrape seed that were pretreated and induced to germinate by exposing them to a 1 ppm solution of GR 24 for 24 hours.

The use of a synthetic cloth instead of a filter paper avoided the development contamination that usually develops on filter paper. Those contamination caused plugging of the paper capilars and dryness. This method is an excellent tool for studing the early phases of broomrape

infection. It can also be used for other studies regarding host: Parasite relationship, herbicide studies etc.

5. To Improve Broomrape Seed Germination.

Considerable progress was achieved by better specifying various conditions for optimal broomrape seed germination in vitro. The study on temperature effect revealed the possibility to study intro-specific differences by conducting germination test under extreme temperatures. The results indicate the possibility to shorten the pretreatment. Period for Egyptian broomrape to 6 days and the need to extend the period to 14 days for Nodding and Crenate broomrape. Also, the possibility to use Ethanol as disinfection agents for broomrape germination studies was verified.

H. Benefit to Agriculture

Tomato is an important vegetable crop worldwide. In recent years, fresh tomatoes have been exported from Israel and have the potential of being an important export crop. Tomato is, however, highly susceptible to broomrape and its yield potentials are limited in areas with broomrape infestation.

Economic damage caused by broomrape to tomato include (a) actual crop loss; (b) losses due to the inability to grow the crop and having to resort to less desirable alternatives; and (c) additional expenses required to control the parasite. None of these have been quantitatively evaluated. Broomrape is a difficult weed to control. Its seeds can persist in the soil for more than 20 years and methods tried for its control have largely failed except for soil fumigation with methyl bromide and soil solarization. A new approach to combat broomrape in tomatoes has been under

investigation at Virginia Polytechnic Institute and State University (USA) and at Agricultural Research Organization, Volcani Center (Israel). It includes screening of tomato varieties for glyphosate, a potent systemic herbicide, and broomrape tolerance/resistance to glyphosate or broomrape or both is to develop commercial varieties of tomato by crop breeding programs so that they can better endure broomrape infestations and allow chemical control measures to work selectively. It is, therefore, obvious that any contribution that can be made to provide the agriculture of Israel and USA with broomrape or glyphosate control in tomatoes and to have them ready in the event of a massive outbreak.

The major screening program conducted at Virginia (USA) and Israel for glyphosate and broomrape tolerance, respectively, has yielded some useful information. First, no resistance to Egyptian broomrape was found. It is possible, although purely speculative, that the strain of the Egyptian broomrape used in the screening program is particularly virulent to tomato and that other populations (strains) of Egyptian broomrape will be less virulent. In fact, the variety PUZ II was reported to be partially resistant to Egyptian broomrape in the U.S.S.R but was susceptible in our experiments. For future work, and in a situation of limited resources, we would not recommend to follow this avenue in searching for a solution to the broomrape problem in tomato.

Secondly, there are some varieties, however that show some tolerance to glyphosate. It is therefore recommended to continue to study those varieties under various regimes of glyphosate application in infested fields. This program is justified by the fact that glyphosate can prove a very useful herbicide for selective control of broomrape in tomato as

indicated by its translocation from the host leaves to broomrape and its accumulation in the parasite.

Developing the method of infecting tomato plants as well as other host plants behind glass in a soilless system is not only interesting and important as a research tool but can be used in applied research of herbicide screening and other control measures.

I. LIST OF PUBLICATIONS

- Foy, C. L. and R. Jacobsohn. 1981. Recent developments relating to the distribution, biology and control of broomrape (Orobanche spp.). Weed Sci. Soc. Amer. Abstr. No. 300.
- Foy, C. L. and R. Jacobsohn. 1983. Screening tomato lines for glyphosate tolerance. Proc. South. Weed Sci. Soc. 36:165.
- Foy, C. L. and R. Jacobsohn. 1984. "Evaluating tomato lines for resistance (tolerance) to glyphosate and/or Orobanche." Presented orally at the Third Intern. Symp. on Parasite Weeds, ICARDA, Aleppo, Syria, May 7-10, 1984. Note: Paper was purged from the bound proceedings after having been accepted.
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Peripheral

- Foy, C. L. 1982. Weed science in perspective. Abstr. 8th National Conference, Weed Sci. Soc. Israel, Hebrew Univ., Rehovot, Israel. Dec. 21, 1981.
- Cooley, W. E. 1985. Comparative studies on the modes of action of SC-0224 and glyphosate. Ph.D. dissertation. Virginia Polytech. Inst. and State Univ., Blacksburg. 85 pp.

APPENDIX

Appendix will be distributed in fewer copies than the report, Copies will be available at:

1. United States-Israel Agricultural Research and Development Fund - BARD
P.O.Box 6, Bet Dagan, 50 250 Israel.
2. BARD-USDA, Federal Building, Hyattsville, Maryland 20782, U.S.A.
3. Regional Plant Introduction Station Iowa State University, Ames Iowa
50011, U.S.A.
4. Tomato Genetics Stock Center, Department of Vegetable Crops, University
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