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Biological control of Egyptian broomrape (*Orobanche aegyptiaca*) using *Fusarium* spp.

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Summary. The broomrape (*Orobanche* spp.) is an obligate holoparasitic weed that causes severe damage to many important vegetable crops. Many broomrape control strategies have been tested over the years. In this investigation, 125 *Fusarium* spp. isolates were recovered from diseased broomrape spikes collected from fields in agricultural areas near Hebron. The pathogenicity of isolates on broomrape was evaluated using an inoculum suspension containing mycelia and conidia. The most effective *Fusarium* isolates significantly increased the dead spikes of broomrape by 33.6–72.7% compared to the control; there was no obvious pathogenic effect on the tomato plants. *Fusarium* spp. isolates Fu 20, 25 and 119 were identified as F. solani, while Fu 30, 52, 59, 87 and 12-04 were *F. oxysporum*. In addition, the two previously known *Fusarium* strains, *F. oxysporum* strain EId (CNCM-I-1622) (Foxy) and *F. arthrosporioides* strain E4a (CNCM-I-1621) (Farth) were equally effective in controlling broomrape parasitizing tomato plants grown in pots, where the dead spikes of broomrape increased by 50.0 and 51.6%, respectively .

Keywords: Biological control, *Fusarium* isolates, *Lycopersicon esculentum*.

Introduction

The broomrape (*Orobanche* spp.) is an obligate holoparasitic weed that causes severe damage to many economic crops in the Mediterranean region and the Middle East (Parker and Riches, 1993). Annual food crop losses due to broomrape infestation are estimated at about $1.3 to 2.6 billion. Egyptian broomrape (*Orobanche aegyptiaca*) together with branched broomrape (*O. ramosa*) infests about 2.6 million ha of solanaceous crops, mainly in the Mediterranean area, North Africa, and Asia (Qasem, 1998; Zehhar *et al*., 2002; Boai *et al*., 2004). In Palestine, broomrape is listed as one of the most harmful weeds and constitute a major constraint to crop production. The control of broomrapes is often difficult for various reasons and numerous control strategies have been tested over the years, with limited effectiveness. The control methods investigated have included changes in agricultural practices, as well as chemical and biological means. Biological methods to control *Orobanche* have been tested by various investigators on different crops but none of them was completely effective (Amsellem *et al*., 2001a). Many pathogenic organisms that seemed to be promising for *Orobanche* control have not been developed to the point of widespread use on account of many problems with formulation, application, and virulence preservation. Hodosy (1981) obtained excellent results in field experiments in Hungary with *Fusarium solani* isolates. A strain of *Rhizoctonia solani* considered to be a biocontrol agent of *Orobanche*,

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has not been seriously considered for mycoherbicidal application because it does not produce spores (Boyette *et al*., 1991, 1996). Bozoukov and Kouzmanova (1994) found that applying conidiospores of *F. lateritium* to tobacco fields during irrigation caused significant control of *Orobanche* sp.

Two very promising isolates, *F. oxysporum* strain E1d (CNCM I-1622) (Foxy) and *F. arthrosporioides* strain E4a (CNCM I-164) (Farth) have been isolated from diseased shoots and their potential for the biocontrol of *Orobanche aegyptiaca* has been studied (Amsellem *et al*., 1999, 2001a,b). Both of these strains infected *O. aegyptiaca, O. cernua,* and *O. ramosa,* but not *O. cumana*. Mycoherbicides developed from these strains strongly affected broomrapes growing on tomato roots, killing 50–100% of the broomrape tubercles; these pathogens had no visible effect on some other vegetable crops (melons, potatoes, tomatoes, peppers, carrots, and celery), a grain legume (chickpeas) or sunflower (Amsellem and Gressel, 2001a). Biocontrol with *Fusarium* spp. often involves phytotoxins such as the fumonisins (Abbas and Boyette, 1992), fusaric acid (Bacon *et al*., 1996) or protein toxins (Bailey *et al*., 2000) which assist in overcoming host defenses, allowing establishment of the pathogen. Thomas *et al*. (1999) reported that conidial suspensions of *F. oxysporum* f. sp. *orthoceras* colonized and infected the seeds of *O. cumana*, a species specific to sunflower.

The objective of the present study was to investigate the efficiency of native *Fusarium* species isolates obtained from diseased broomrape spikes in the control of tomato broomrape (*Orbanche aegyptiaca*), comparing them with the effectiveness of the two previously identified control strains, Foxy and Farth.

Materials and methods

Isolation of *Fusarium* species

In the summer of 2003 and 2004, during a field survey of broomrape, 135 samples of diseased broomrape spikes were collected from various vegetable fields around Hebron. Forty-four samples were collected from fields of naturally-infested tomato plants growing under rain-fed conditions, sixty from drip-irrigated fields, and thirty-one from fields of eggplant, cabbage, cauliflower, salvia, sunflower and chickpea. Diseased tubercles and broomrape spikes were cut into 3–4 mm pieces, surface–sterilized by immersion in a 1% sodium hypochlorite solution for 4 min, and rinsed three times with sterile distilled water. Three pieces per Petri dish were placed in 90 mm plastic Petri dishes containing Komada medium (Komada, 1975). The medium was dissolved and autoclaved (122°C for 15 min). The selective medium was poured into 90 mm plates (20 ml per plate). After ten days, single isolates growing as fungal hyphae out of the broomrape pieces were subcultured on potato dextrose agar (PDA) medium amended with 250 mg l^{-1} chloramphenicol. Single spore cultures of the isolates were subcultured, and one of the growing colonies was used to inoculate several dishes. The fungal isolates were later identified taxonomically (Burgess *et al*., 1994).

Pathogenicity bioassays

The *Fusarium* isolates that recorded the highest mycelium growth rate and the highest conidial production (Fu 8-04, 12, 12-04, 20, 25, 52, 59,77, 87, 100, 116, 2, 2-4, 4/2, 5, 5-04, 6, 14, 16, 23, 30, 45, 53, 75, 115, 119 and 123) were selected for the pathogenicity studies, in addition to the two well known strains parasitizing broomrape, Foxy and Farth, which had been previously isolated from a melon field heavily infested with broomrape and obtained from J. Gressel, Laboratory of The Weizmann Institute of Science, Rehovot, Israel and deposited with the Collection Nationale de Cultures de Microorganismes (CNCM), Institut Pasteur, Paris, France. The fungal biomass was prepared by gently transferring a 5-mm mycelium plug from two-week-old fungal colonies of *Fusarium* isolates to each of three autoclaved (200 ml) flasks containing 100 ml potato dextrose broth amended with 250 mg l^{-1} chloramphenicol. The flasks were shaken for 14 days at 25°C with a 14-h daylight. Spores from cultures were washed free of mycelia through Miracloth (Calbiochem®, San Diego, CA, USA), centrifuged (at 2308 *g* [RCF] for 20 min), and the supernatant was decanted. The spores were resuspended in sterile water, centrifuged again, decanted, and resuspended as above. Spore concentrations were determined with a haemocytometer. The mycelia pads were washed three times with sterilized, deionized and distilled water, and weighed to obtain their fresh weight. The inoculum of each isolate was a conidium-mycelium suspension (CMS)

prepared by incorporating both fungal mycelium and fungal conidia which were separately suspended in distilled water. A mixture of 5 g fungal mycelium with 5×10^8 conidia was then suspended in 15 ml 1 M sucrose. The suspension was homogenized by a Polytron PT 1200C homogenizer (Kinematica®AG, Luzern, Switzerland) for 60 seconds at 5000 rpm. The experiment was conducted in a completely randomized design and included four replicates (pots); each pot was 4 l in size and contained 3 kg soil. Treatments included growing tomato plants in broomrape-free soil (To); in soil infested with broomrape at an inoculum concentration of 40 mg broomrape seeds kg^{-1} soil (T1); and in soil infested with broomrape plus one of the *Fusarium* isolates (T2). The inoculum of each *Fusarium* isolate was calibrated to a concentration of 10^8 conidia and 0.5 mg mycelium g^{-1} soil. Four-week-old tomato plants growing in pots were watered with 50 ml of each CMS followed by 100 ml of water for the T2 treatment.

The plants in To and T1 were watered with 150 ml water per pot. Plants were then incubated under greenhouse conditions at 25°C and watered as necessary. After 8 weeks, the number and weight of all spikes, dead spikes and viable spikes were recorded. In addition, the disease rate, and the fresh and dry weights of the tomato plants were recorded. The pathogenicity bioassays of the *Fusarium* isolates were carried out in two experiments; the first included testing 13 isolates and the second included 18 isolates (Table 1). Another experiment was designed to include the most effective *Fusarium* isolates (Foxy, Farth, Fu 12-04, 20, 25, 30, 52, 59, 87, and 119) and the same parameters were measured and recorded.

Identification of *Fusarium* isolates

The most promising *Fusarium* isolates (Fu 30, 119, 59, 20, 52, 12-04, 87 and 25) were identified according to Burgess *et al*. (1994). A suspension of conidia was prepared by placing a disc of a one-week-old *Fusarium* isolate culture in 10 ml sterile distilled water in a test tube; the suspension was vortexed for 60 seconds at 5000 rpm. The conidia were separated by filtering the suspension

Table 1. Effect of *Fusarium* spp. isolates on tomato fresh and dry weight.

First experiment		
Isolate	Weight (g)	
	Fresh	Dry
CK+Br	55 c*	8.25 c
CK-Br	149 a	22.35 a
Farth	87 b	13.05 b
Foxy	81 c	12.15 c
Fu 20	61 c	9.15 c
Fu 25	63 c	9.45 c
Fu 52	105 b	15.75 b
Fu 59	89 b	13.35 b
Fu 77	62 c	9.3 c
Fu 87	118 a	17.7 a
Fu 100	62 c	9.3 c
Fu 112	84 c	12.6 c
Fu 116	79 c	11.8 c
Fu 8-04	94 b	14.1 b
Fu 12-04	79 c	11.85 c

Second experiment		
Isolate	Weight (g)	
	Fresh	Dry
CK+Br	112 c	19.04 c
CK-Br	238 b	40.46 b
Foxy	259 b	44.03 b
Farth	232 b	39.44 b
Fu 2	308 a	52.36 a
Fu 4/2	193 b	32.8 b
Fu 5	300 a	51 a
Fu 6	266 b	45.22 b
Fu 14	115 c	19.55 c
Fu 16	115 c	19.55 c
Fu 23	126 c	21.42 c
Fu 30	291 a	49.47 a
Fu 45	258 b	43.86 b
Fu 53	281 a	47.8 a
Fu 75	203 b	34.5 b
Fu 115	214 b	36.38 b
Fu 119	377 a	64.1 a
Fu123	167 b	28.39 b
Fu 2-04	238 b	40.46 b
Fu 5-04	276 a	46.9 a

[a] Means followed by the same letter in each column are not significantly different according to Fisher's LSD test ($P\leq0.05$).

through Miracloth. The conidial suspension (200 µl) was seeded on 90-mm PDA plates, with three replicates for each isolate. The plates were incubated at 25ºC. After 3 days, a single germinated conidium was removed using a fine sterile needle and transferred to a new PDA plate. Inoculated plates were incubated at 25ºC and after three days the plates were exposed to natural light at 25ºC. After one week, conidial suspensions were prepared and three plates were inoculated centrally with a single conidium of each isolate. The plates were incubated at 25ºC and colony diameters were measured after three days. These plates were then transferred and incubated under natural light for three weeks. The following parameters were evaluated and studied for taxonomic differentiation: mycelium diameter, pigmentation and color produced by the isolate on PDA medium, shape of micro and macroconidia and presence or absence of chlamydospores.

Statistical analysis

The data of dead broomrape spikes and tomato fresh and dry weights were analyzed statistically according to Fisher LSD test (P≤0.05) using Sigma Stat® software.

Results

Pathogenicity bioassays

The first experiment on *Fusarium* spp. pathogenicity showed that ten isolates significantly ($P \leq 0.05$) controlled broomrape of tomato plants in pots (Fig. 1). Dead broomrape spikes increased by 37.6–56.9% compared with the control. In the second experiment, five isolates significantly ($P \leq 0.05$) controlled broomrape and the percentage of dead spikes increased by 67.3 to 100% compared with the control (Fig. 1).

The evaluation of the most promising *Fusarium* spp. isolates was repeated, and the results confirmed that the isolates Farth, Foxy, Fu 30, 87, 20, 119, 59 and 52 caused the highest percentages of dead spikes, ranging from 33.6 to 72.7%, as compared with the control (Fig. 2). Diseased broomrape spikes showed necrotic black lesions.

In general, the tomato plant fresh weights increased when the *Fusarium* isolates were added. In the first experiment, the tomato plant fresh weight increased significantly from 58 to 114% and in the second experiment by 49–237% compared with the control (Table 1). None of the *Fusarium* isolates had any negative effect on the tomato plants.

Identification of *Fusarium* isolates.

According to the taxonomic keys (Burgess *et al*., 1994), isolates Fu 20, 25 and 119 were identified as *Fusarium solani*; their colony diameters after three days were 1.8, 2.4 and 2.1 cm, respectively. The color of the colonies growing on PDA medium was pink for Fu 20, dark pink for Fu 25, and red for Fu 119. The mycelium of all isolates was segmented; microconidia were oval for Fu 20, obovoid or oval for Fu 25, and oval for Fu 119; macroconidia of all the three isolates had their dorsal side more curved than their ventral side, and chlamydospores were found with Fu 20 and Fu 25, but not with Fu 119.

Isolates Fu 30, 52, 59, 87 and 12-04 were identified as *F. oxysporum*. Their colony diameters after three days of single conidial culturing were 2.1, 1.0, 2.1, 2.1 and 2.2 cm respectively. The color of the colonies grown on PDA were peach or pale orange for Fu 30, rose red for Fu 52, dark pink for Fu 59, red for Fu 87 and rose red for Fu 12-04. The mycelium of all isolates was segmented; microconidia were obovoid with a truncate base for Fu 30, oval for Fu 52, obovoid with a truncate base for Fu 59, and obovoid for Fu 87 and Fu 12-04. Fu 30 and Fu 87 macroconidia were slender and straight, while for Fu 52, Fu 59, and Fu 12-04 the dorsal side of macroconidia was more curved than the ventral side. Chlamydospores were found in Fu 52, Fu 59 and Fu 12-04, but not in Fu 30.

Discussion

Eight Fusarium isolates (Fu 20, 30, 52, 59, 87, 119, Farth and Foxy) showed high efficiency in controlling broomrape of tomato plants growing in pots. However, isolates showed variation in pathogenicity against broomrape. Treating the soil with the isolates Fu 30 and 119, induced a general increase in fresh and dry weights of tomato plants, probably as a result of the control of broomrape and/or competition of the applied isolates with other minor soilborne pathogens for available nutrients. However, isolates Fu 20, 52, 59, 87, Farth and Foxy had no positive effect on tomato fresh

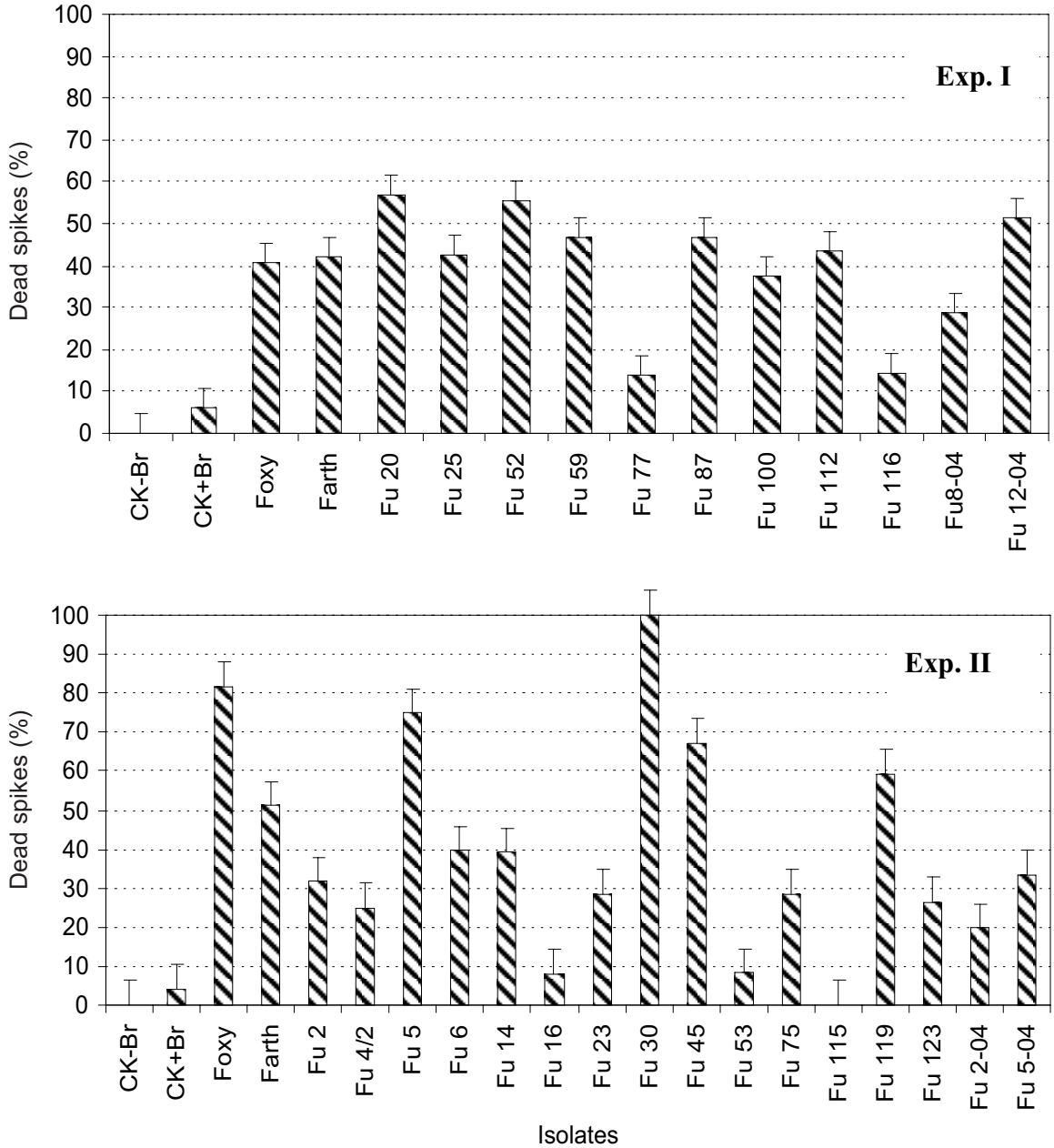

Fig. 1. Effect of *Fusarium* spp. isolates in pathogenicity tests on broomrape of tomato plants grown in pots.

and dry weights. It is worth mentioning as well that the applied *Fusarium* isolates were not pathogenic to the tomato plants. The isolates Fu 30, 52, 59, 87 and 12-04, isolated from rain-fed tomato fields, were later identified as *F. oxysporum*, and isolates Fu 20, 25 and 119, isolated from irrigated tomato fields, were later identified as *F. solani*. *F. oxysporum* isolates were generally more efficient in the control of broomrape than *F. solani* isolates; *F. oxysporum* isolates increased the percentage of broomrape dead spikes by 16.2–72%, and *F. solani* isolates by 16.6–57.1% (Fig. 2, Table 2).

In this connection, many investigators (Amsellem *et al*., 1999, 2001; Cohen *et al*., 2002) reported

that Foxy and Farth were pathogenic to *O. aegyptiaca*. Boari and Vurro (2004) found that a strain of *F. oxysporum* and a strain of *F. solani* strongly reduced the number and the weight of emerging broomrape. Thomas *et al*. (1998, 1999) reported that some isolates of *F. oxysporum* inhibited *O. cumana* seed germination and decreased the number of broomrape attachments on sunflower roots. Furthermore, Bedi and Donchev (1991) reported that *F. oxysporum* f. sp. *orthoceras* is a potential agent for the biological control of *O. cumana* in sunflower.

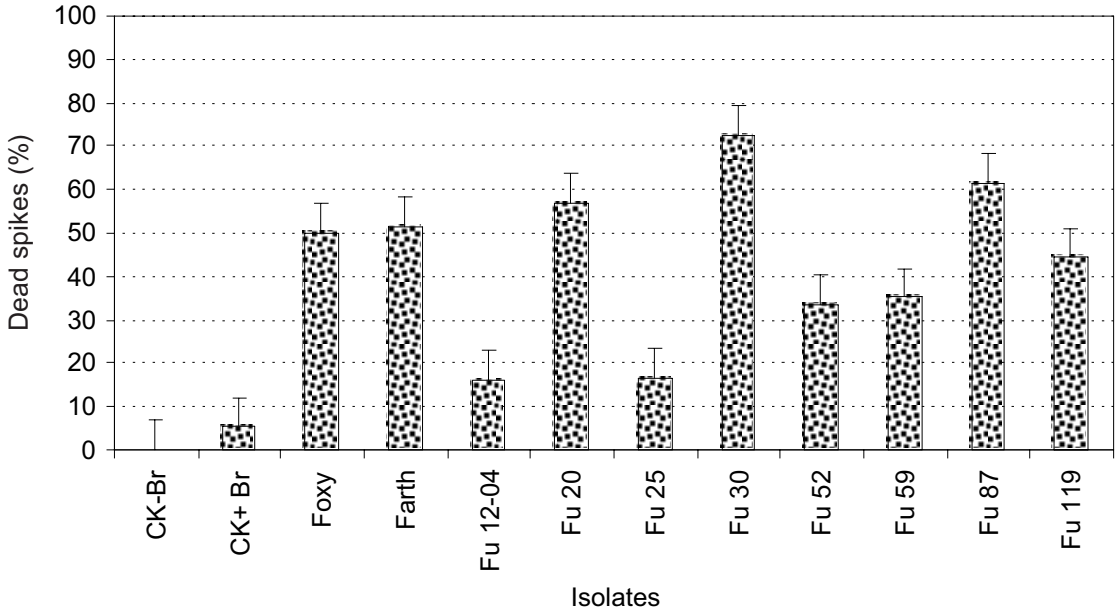

Fig. 2. Effect of pathogenicity tests with the selected promising *Fusarium* spp. isolates on broomrape of tomato plants grown in pots. The pots contained *Fusarium* infested soil.

Table 2. Effect of the most promising *Fusarium* spp. isolates on the number and weight of broomrape dead spikes, tomato fresh and dry weight.

Isolate	Broomrape dead spikes[a]		Tomato plant weight (g)[a]	
	Dead (%)	Weight (g)	Fresh	Dry
CK-Br	0.0 b	0 b	300 a	45 a
CK+ Br	5.4 b	0 b	168 b	25.2 b
Fu 12-04	16.2 b	1.3 b	206 b	30.9 b
Fu 20	57.1 a	28 a	123 c	18.45 c
Fu 25	16.6 b	1.3 b	165 b	24.75 b
Fu 30	72.7 a	9.3 b	208 b	31.2 b
Fu 52	33.6 a	9.3 b	140 b	21 b
Fu 59	35.2 a	6.3 b	114 c	17.1 c
Fu 87	61.5 a	18 a	104 c	15.6 c
Fu 119	44.3 a	6.3 b	221 a	33.15 a
Foxy	50.0 a	3.8 b	189 b	28.35 b
Farth	51.6 a	14 a	159 b	23.85 b

[a] Means followed by the same letter in each column are not significantly different according to Fisher's LSD test ($P \leq 0.05$).

Diseased broomrape spikes showed water-soaked necrotic black lesions before death. These symptoms may be related to the hydrolysis of broomrape tissues and starch depletion out of the infected tissues; the sugars derived from the starch may be used by the pathogen as energy to mobilize a rapid response to infection. Other modes of action have been suggested by several investigators. *F. oxysporum* often produces phytotoxins such as fumonisins (Abbas and Boyette, 1992), fusaric acid (Bacon *et al*., 1996) and protein toxins (Bailey *et al*., 2000), which assist in overcoming the host defenses, allowing establishment of the pathogen. Amsellem *et al*. (2001) reported that Foxy and Farth may deplete starch out of infected *Orobanche*. In addition, Desjardins and Hohn (1997) stated that *Fusarium* strains produced toxins such as fusaric acid, fuminisins, beauvericin, enniatin, moniliformin and trichothecenes, many of which have a phytotoxic or herbicidal effect.

In conclusion, *Fusarium oxysporum* Fu 30, Fu 87 and Foxy, *Fusarium solani* Fu 20, and Farth gave promising results in controlling broomrape of tomatoes. Further field trials are needed to confirm the efficacy of these strains under common agricultural practices and to clarify the mode of action of these strains against various *Orobanche* species.

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