

Interaction between *Glomus mosseae* and *Erwinia carotovora* and its effects on the growth of tomato plants

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SUMMARY

The effect of inoculation with *Erwinia carotovora* pv. *carotovora* on tomatoes with or without VA mycorrhizal infection by *Glomus mosseae* was studied in a greenhouse trial. *E. carotovora* decreased the growth of nonmycorrhizal tomato plants, but neither growth nor the percentage of total root length with VA infection were reduced in mycorrhizal plants. Protection of tomato against the pathogen and reduction in the number of colony-forming units of *E. carotovora* in the rhizosphere of mycorrhizal plants were independent of concentration of P in the plant and the timing of inoculation with the two microorganisms. The possible mechanisms of the resistance induced in tomato plants by *G. mosseae* against *E. carotovora* are discussed.

Key words: Tomato; *Glomus mosseae*; *Erwinia carotovora*; VA mycorrhizas; plant pathogenic bacteria.

INTRODUCTION

Studies of interactions between vesicular-arbuscular (VA) mycorrhizal fungi and pathogenic organisms such as nematodes and fungi (Dehne, 1982; Hussei & Roncadori, 1982), have shown that the protective effects of VA mycorrhizal fungi against pathogen and disease development can be attributed to better plant nutrition, enhanced growth, and, occasionally specific, physiological stimulation in mycorrhizal plants (Dehne, 1982). In most studies, plants have been inoculated with VA mycorrhizas before exposure to the pathogen (Bagyaraj, 1984). Induced resistance depends on the time elapsed between inoculation of the VA fungus and the pathogen (Bartschi, Gianinazzi-Pearson & Veigh, 1981). However, under natural conditions both microorganisms frequently occur simultaneously in the rhizosphere of the same plant.

The activity of rhizosphere-inhabiting microorganisms exerts a significant effect upon plant health. Quantitative changes in the bacterial population in the rhizosphere owing to inoculation with VA mycorrhizal fungi have been reported (Bagyaraj & Menge, 1978). However, there are only two reports in which plant susceptibility to bacterial pathogens (*Pseudomonas* spp.) was studied in relation to the incidence of VA mycorrhizal fungi in roots

(Weaver & Wehunt, 1975; Halos & Zorrilla, 1979). The pectolytic and other macerating enzymes produced by *Erwinia carotovora* pv. *carotovora* result in a loss of structural integrity in host tissue and a characteristic soft rot. Although generally associated with vegetables, *E. carotovora* is also naturally pathogenic to a wide range of ornamental plants and field crops (Cother & Sivasithamparam, 1983).

The purpose of this study was to determine the relationships between *Glomus mosseae* and *E. carotovora* and to examine some of the possible mechanisms involved in the resistance induced in VA mycorrhizal tomato plants grown in two soil concentrations of 1:9 and 1:1 (v/v) of soil:sand.

MATERIALS AND METHODS

Experiments were carried out on plants grown in 300 ml capacity open pots of soil collected from Granada Province, Spain. The soil was a 'reddish brown-calcareous' type, pH 7.6 (for full details see Barea, Escudero & Azcon-Aguilar, 1980). It was steam-sterilized and mixed with sterilized sand in the proportion 1:9 (v/v) or 1:1 (v/v) of soil:sand.

Tomato (*Lycopersicon esculentum* Mill. cv. Marglobe) was used as the test plant. Seeds were sown in moistened sand. After 2 weeks, seedlings were transplanted to the pots and grown under green-

house conditions (natural light was supplemented by Sylvania incandescent and cool-white lamps, 400 nmol m⁻² s⁻¹, 400–700 nm; with a 16/8 h light/dark cycle at 25/19 °C and 50% relative humidity). Plants were watered from below using a capillary system and fed with a phosphate-free nutrient solution (2 ml week⁻¹) (Smith & Smith, 1981).

The VA inoculum consisted of 5 g of rhizosphere soil from maize plant pot cultures of a isolate of *Glomus mosseae* which contained spores, mycelium and infected root fragments. Uninoculated plants were given filtered leachings from the inoculum soil; the filtrate contained common soil microorganisms, but no propagules of *G. mosseae*.

Erwinia carotovora, isolate no. 225, was obtained from C.I.B. (Madrid, Spain). An aqueous suspension in sterile distilled water containing approximately 2.5 × 10⁸ bacteria ml⁻¹ was prepared from cultures grown on nutrient agar (Oxoid) for 47 h at 27 °C. The plants were inoculated by pouring 10 ml of the bacterial suspension onto the soil surface. Control plants received 10 ml distilled water.

Four treatments were used in all experiments: (1) uninoculated control, (2) inoculated with *E. carotovora*, (3) inoculated with *G. mosseae*, and (4) inoculated with both *E. carotovora* and *G. mosseae*. Plants were inoculated with *G. mosseae* at the time of transplanting or after 3 weeks of growth. In all experiments the bacterium was inoculated after 3 weeks of plant growth.

During the experiments, in order to evaluate the population of inoculated *E. carotovora*, rhizosphere soil was sampled at 15 d intervals, as described by Ocampo, Barea & Montoya (1975). About 1.5 g of rhizosphere soil was taken from each of the experimental pots. Then 10-fold dilution series were prepared for each sample. The first sample was taken 5 d after bacterial inoculation. The number of *E. carotovora* colony-forming units (c.f.u.s) in suitable dilutions of such samples, taken from the five replicate pots of each treatment, were counted on a differential solid medium (Logan, 1966) containing a 2% pectate overlay. Rhizosphere soil was quantified as follows: soil from dilutions to 10⁻¹ and 10⁻² was recovered, dried at 105 °C and weighed. The number of c.f.u.s was expressed per g of dry rhizosphere soil.

Plants were harvested after 8 weeks and the amount of dry matter determined. After harvesting, parts of the root system from each of the five replicate pots were cleared and stained (Phillips & Hayman, 1970) and the percentage of total root length which was infected with VA mycorrhiza was measured by the gridline intersect method (Giovannetti & Mosse, 1980).

The N, P and K concentrations of plant shoots were determined by methods of the Estacion Experimental del Zaidin using a Technicon autoanalyser.

Percentages of root length infected by VA my-

corrhiza and N, P and K concentrations of plant shoots were transformed using $y = \arcsin \sqrt{1/x}$ before statistical analysis.

RESULTS

The percentage of root length of tomato plants infected with *G. mosseae* was similar in all treatments tested (Table 1), irrespective of the presence or absence of *E. carotovora* and the soil:sand concentration.

Plant dry weights were lower when plants were grown in pots with a 1:9 (v/v) mixture (Table 2a) than with a 1:1 (v/v) mixture of soil:sand (Table 2b). Infection with mycorrhizal fungi increased shoot dry weights at both soil concentrations. Inoculation of nonmycorrhizal plants with *E. carotovora* depressed shoot and root dry matter, the stems became light brown and the leaves chlorotic. In plants inoculated with both organisms, mycorrhizal infection overcame any growth reduction caused by the bacteria; shoot weights were higher than in mycorrhizal-only plants regardless of time of inoculation, in plants at high soil concentration (Table 2b) and at the low soil concentration were similar to plants inoculated with both microorganisms at the same time (Table 2a).

The number of c.f.u.s g⁻¹ rhizosphere soil decreased throughout the experiments, although numbers were consistently lower in mycorrhizal than non-mycorrhizal plants (Table 2). Populations of *E. carotovora* were lower in the rhizosphere of plants grown in pots with the lower concentration of soil than in pots with the higher concentration.

Infection of plants with VA mycorrhizal fungi increased the concentration of phosphorus (% P in dry matter) of shoots of plants at both soil concentrations (Table 3). Bacterial inoculation had no significant effect on the phosphorus concentration of shoots. The concentration of P in shoot dry matter in dually inoculated plants was similar to mycorrhizal-only plants. When *E. carotovora* was inoculated in absence of *G. mosseae*, % N in shoot dry matter was higher than in the other treatments regardless of soil concentration. The concentration of K in shoots was similar after all treatments.

DISCUSSION

E. carotovora depressed the growth of dry matter of nonmycorrhizal tomato plants, but neither the dry weight of VA mycorrhizal plants, nor the percentage of root length having VA infection, were reduced by the pathogenic bacterium. In experiments with other pathogenic organisms, high population density could swamp the protection of plants afforded by VA mycorrhizas (Davis & Menge, 1981). However, in the present case, the number of bacteria present in the rhizosphere of VA mycorrhizal tomatoes did not

Table 1. Percentage of root length infected by VA mycorrhiza, and dry weight of shoots and roots, of tomato plants grown in soil:sand mixture either (a) 1:9 (v/v) or (b) 1:1 (v/v) and inoculated or uninoculated with *Glomus mosseae* in presence or absence of *Erwinia carotovora*

Inoculation time	Treatments	Plant dry weight (mg)		Root length infected (%)	TD*
		Shoots	Roots		
(a) Soil:sand mixture (1:9)					
<i>E. carotovora</i> inoculated at the same time as <i>G. mosseae</i>	Control	105	92	—	—
	<i>E. carotovora</i>	60	52	—	—
	<i>G. mosseae</i>	152	121	40	0.68
	<i>G. mosseae</i> plus <i>E. carotovora</i>	158	124	31	0.58
	LSD (5%)	31	28	—	0.10
<i>E. carotovora</i> inoculated 3 weeks after <i>G. mosseae</i>	Control	122	121	—	—
	<i>E. carotovora</i>	62	49	—	—
	<i>G. mosseae</i>	175	134	57	0.85
	<i>G. mosseae</i> plus <i>E. carotovora</i>	336	124	63	0.91
	LSD (5%)	30	35	—	0.10
(b) Soil:sand mixture (1:1)					
<i>E. carotovora</i> inoculated at the same time as <i>G. mosseae</i>	Control	217	75	—	—
	<i>E. carotovora</i>	101	42	—	—
	<i>G. mosseae</i>	290	165	41	0.69
	<i>G. mosseae</i> plus <i>E. carotovora</i>	470	160	48	0.76
	LSD (5%)	62	55	—	0.10
<i>E. carotovora</i> inoculated 3 weeks after <i>G. mosseae</i>	Control	280	89	—	—
	<i>E. carotovora</i>	100	50	—	—
	<i>G. mosseae</i>	600	140	56	0.84
	<i>G. mosseae</i> plus <i>E. carotovora</i>	720	452	62	0.90
	LSD (5%)	102	70	—	0.09

Each figure is the mean for five pots.

* TD, Transformed data, percentage transformed using $y = \arcsin \sqrt{1/x}$.

Table 2. Colony-forming units (c.f.u.s) of *Erwinia carotovora* from the rhizosphere (g^{-1} dry weight of soil) of tomato plants grown in soil:sand mixture of either (a) 1:9 (v/v) or (b) 1:1 (v/v) and inoculated or uninoculated with *Glomus mosseae*

Inoculation time	Treatments	C.f.u. (hundreds g^{-1} dry rhizosphere soil)		
		5	20	35
(a) Soil:sand mixture (1:9)				
<i>E. carotovora</i> inoculated at the same time as <i>G. mosseae</i>	<i>E. carotovora</i>	3420	2352	1421
	<i>E. carotovora</i> plus <i>G. mosseae</i>	2252	854	303
	LSD (5%)	341	218	242
<i>E. carotovora</i> inoculated 3 weeks after <i>G. mosseae</i>	<i>E. carotovora</i>	2452	1954	1093
	<i>E. carotovora</i> plus <i>G. mosseae</i>	2583	870	671
	LSD (5%)	364	231	264
(b) Soil:sand mixture (1:1)				
<i>E. carotovora</i> inoculated at the same time as <i>G. mosseae</i>	<i>E. carotovora</i>	1342	1243	575
	<i>E. carotovora</i> plus <i>G. mosseae</i>	1253	259	285
	LSD (5%)	104	284	201
<i>E. carotovora</i> inoculated 3 weeks after <i>G. mosseae</i>	<i>E. carotovora</i>	1665	1430	678
	<i>E. carotovora</i> plus <i>G. mosseae</i>	1642	203	326
	LSD (5%)	56	216	209

Each figure is the mean for five pots.

Table 3. Concentration of N, P and K (% dry matter) in shoots of tomato plants grown in a soil:sand mixture of either (a) 1:9 (v/v) or (b) 1:1 (v/v) and inoculated or uninoculated with *Glomus mosseae* in presence or absence of *Erwinia carotovora*

Inoculation time	Treatments	Concentration					
		N		P		K	
		% Dry matter	TD*	% Dry matter	TD	% Dry matter	TD
<i>(a)</i> Soil:sand mixture (1:9)							
<i>E. carotovora</i> inoculated at the same time as <i>G. mosseae</i>	Control	1.03	0.10	0.07	0.026	2.83	0.16
	<i>E. carotovora</i>	3.00	0.17	0.07	0.026	2.44	0.15
	<i>G. mosseae</i>	1.10	0.10	0.10	0.031	2.79	0.16
	<i>G. mosseae</i> plus <i>E. carotovora</i>	1.73	0.13	0.11	0.033	2.52	0.15
	LSD (5%)	—	0.01	—	0.002	—	0.01
<i>E. carotovora</i> inoculated 3 weeks after <i>G. mosseae</i>	Control	1.00	0.09	0.06	0.024	2.52	0.15
	<i>E. carotovora</i>	3.61	0.19	0.07	0.026	2.59	0.16
	<i>G. mosseae</i>	1.09	0.10	0.09	0.029	2.52	0.15
	<i>G. mosseae</i> plus <i>E. carotovora</i>	1.59	0.12	0.10	0.031	2.54	0.15
	LSD (5%)	—	0.01	—	0.001	—	0.01
<i>(b)</i> Soil:sand mixture (1:1)							
<i>E. carotovora</i> inoculated at the same time as <i>G. mosseae</i>	Control	1.20	0.10	0.14	0.037	2.80	0.16
	<i>E. carotovora</i>	2.00	0.14	0.15	0.038	2.15	0.15
	<i>G. mosseae</i>	1.35	0.11	0.18	0.042	2.89	0.17
	<i>G. mosseae</i> plus <i>E. carotovora</i>	1.21	0.10	0.18	0.042	2.52	0.15
	LSD (5%)	—	0.01	—	0.002	—	0.10
<i>E. carotovora</i> inoculated 3 weeks after <i>G. mosseae</i>	Control	1.21	0.11	0.15	0.038	2.42	0.15
	<i>E. carotovora</i>	2.04	0.14	0.16	0.039	2.54	0.15
	<i>G. mosseae</i>	1.20	0.10	0.20	0.044	2.24	0.14
	<i>G. mosseae</i> plus <i>E. carotovora</i>	1.04	0.10	0.21	0.045	2.03	0.14
	LSD (5%)	—	0.10	—	0.002	—	0.01

* See Table 1 for explanation.

reduce plant growth. For several other plant pathogens, moderate levels of soil P, not inhibitory to mycorrhizal activity, had the same effect as VA mycorrhizas in reducing the severity of effects (Davis & Menge, 1980). However, the higher P concentration in leaves of tomato following an increase in available P in the soil, was not associated with a decrease in the population of *E. carotovora*. The protection of tomato by *G. mosseae* and the reduction in the number of c.f.u.s of *E. carotovora* were observed irrespective of P concentrations in plants.

In our experiments, VA mycorrhizal infection protected tomato plants against *E. carotovora* when both microorganisms were inoculated at the same time, i.e. when the effect of the mycorrhiza is unlikely to have been via host nutrition. This suggests there was, possibly, a direct interaction between the mycorrhizal fungus and the pathogen in the extramatrical phase of the former. Similar suggestions have been made for other root pathogens (Caron, Fortin & Richard, 1985).

Further investigations should examine the pos-

sible competition for nutrients between organisms, the production of inhibitory compounds by VA mycorrhizal fungi or their host, and the ecological conditions that could enhance such advantages. The diversity of interactions between VA mycorrhizas and plant pathogenic microorganisms means, however, that each combination may be unique and generalizations regarding such interactions must be made with caution (Dehne, 1982; Bagyaraj, 1984).

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