

EFFECT OF VA MYCORRHIZAL INFECTION OF TOMATO ON DAMAGE CAUSED BY *PSEUDOMONAS SYRINGAE*

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Summary—The growth of nonmycorrhizal infected tomato was decreased by *Pseudomonas syringae*. However, in mycorrhizal plants neither growth nor percentage of VA infection was negatively affected by the pathogenic bacteria. The protection of tomato by *Glomus mosseae* against the pathogen and the reduction in the number of colony-forming units of *P. syringae* in the rhizosphere of mycorrhizal plants was independent of P plant concentration and timing of inoculation with the two microorganisms.

The possible mechanisms of the protection of tomato plants by *G. mosseae* against *P. syringae* are discussed.

INTRODUCTION

Rhizosphere-inhabiting microorganisms exert a significant effect upon plant health. Several investigations have shown that vesicular-arbuscular (VA) mycorrhizas affect the severity of growth effects induced in a host plant by pathogenic organisms (Dehne, 1982). Most studies have been focused on the effect of VA mycorrhizas on nematodes, pathogenic fungi and viruses, but there are only two reports in which plant susceptibility to bacterial pathogens was studied in relation to the incidence of VA fungi (Bagyaraj, 1984), and different results have been observed. The susceptibility of peach plants to the bacterial canker caused by *Pseudomonas syringae* was not affected by VA mycorrhizal infection (Weaver and Wehnt, 1975), but a reduction in the severity of bacterial wilt of VA mycorrhizal infected tomatoes caused by *P. solanacearum* was found (Halos and Zorrilla, 1979).

Interaction studies between VA mycorrhizal fungi and pathogenic organisms have shown that the effects of VA mycorrhizal fungi on pathogen and disease development can be attributed to better nutrition, enhanced plant growth and physiological stimulation in the mycorrhizal plants (Dehne, 1982). Thus, the resistance to pathogens induced by VA mycorrhizas seems to depend on the time elapsed between inoculation of the VA fungus and of the pathogen (Bartschi *et al.*, 1981). However, both, VA fungi and pathogens, frequently occur simultaneously in the rhizosphere of the same plant.

Our purpose was to determine the relationships between *Glomus mosseae* and *P. syringae* and to examine some of the possible mechanisms involved in the protection of tomato plants by VA mycorrhizas.

MATERIALS AND METHODS

The experiments were carried out in open roots (300 ml) of soil collected from Granada Province, Spain. The soil was a "reddish brown-calcareous"

type, pH 7.6 (for full details see Barea *et al.*, 1980). It was steam-sterilized and mixed with sterilized sand to the proportion 1:9 (v/v) or 1:1 (v/v) of soil:sand.

Tomato (*Lycopersicon esculentum* Mill. cv. Marglo) was used as the test plant. Seeds were sown in moistened sand, and 2-week-old seedlings were transplanted to the pots and grown under greenhouse conditions; supplementary light (Silvania incandescent and cool-white lamps, 400 nmol m⁻² s⁻¹ 400–700 nm), 16–8 h light–dark cycle, 25–19°C, 50% r.h. Plants were watered from below using a capillary system and fed with a phosphate-free nutrient solution (2 ml wk⁻¹) (Smith and 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. Soil filtrate (Whatman No. 1 filter paper) from the rhizosphere of mycorrhizal maize plants was added to the VA uninoculated treatments. The soil filtrate contained common soil microorganisms with no propagules of Endogonaceae.

The *Pseudomonas syringae* isolate No. 3053 was obtained from C.I.B. (Madrid, Spain). An aqueous suspension in sterile distilled water containing approx. 2.5 × 10⁷ bacteria ml⁻¹ was prepared from cultures grown in nutrient agar (g⁻¹: Difco nutrient agar 23; sucrose 10) 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: (1) uninoculated control; (2) inoculated with *P. syringae*; (3) inoculated with *G. mosseae*; and (4) inoculated with both *P. syringae* and *G. mosseae* were used in all experiments. Plants were VA inoculated at the time of transplanting or after 3 weeks of growth. In all experiments pathogenic bacteria were inoculated after 3 weeks of plant growth.

During the experiments rhizosphere soils were sampled every 15 days (Ocampo *et al.*, 1975). About 1.5 g of rhizosphere soil was taken from each of the experimental pots. Then 10-fold dilution series were

Table 1. VA root length infection and dry weight (shoot and roots) of tomato plants grown in soil-sand mixture (1:9 v/v) inoculated or uninoculated with *G. mosseae* in presence or absence of *P. syringae*

Inoculation time	Treatments	Plant dry wt (mg)		% Root length infected
		Shoots	Roots	
<i>P. syringae</i> inoculated at the same time as <i>G. mosseae</i>	C	110	102	
	P	35	32	
	M	168	131	56
	M + P	137	96	69
<i>P. syringae</i> inoculated 3 weeks after <i>G. mosseae</i>	LSD 5%	25	39	11
	C	122	121	
	P	43	40	
	M	175	138	57
	M + P	450	241	63
	LSD 5%	36	43	8

C = Uninoculated control; P = plants inoculated with *P. syringae*; M = plants inoculated with *G. mosseae*. Each figure is the mean for five pots.

prepared for each sample. The first sample was taken 5 days after bacterial inoculation.

The number of colony forming units (cfu) of *P. syringae* in suitable dilutions of such samples, taken from the five replicate pots of each treatment, were counted on a differential solid medium (Leben, 1986). To provide a basis for countings, rhizosphere soil was quantified as follows: in 10^{-1} and 10^{-2} dilutions, soil was recovered, dried at 105°C and weighed. The number of cfu was expressed g^{-1} dry rhizosphere soil.

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

RESULTS

Growth responses of tomato to inoculation with *G. mosseae* or *P. syringae* or both are shown in Table 1. Inoculation with mycorrhizal fungi improved shoot growth in both soil concentrations.

The VA mycorrhizal root length infection of tomato was similar in all treatments tested (Table 1) regardless of the presence or absence of *P. syringae*.

Inoculation of nonmycorrhizal plants with *P. syringae* depressed shoot and root growth. Mycorrhizal inoculation overcame any growth depressions caused by the bacteria in dually inoculated plants (Table 1).

Shoot weight of plants inoculated with both microorganisms at the same time was similar to plants inoculated only with *G. mosseae*. But when *P. syringae* was inoculated 3 weeks after *G. mosseae*, dually infected plants were heavier than mycorrhizal-only plants.

The number of cfu g^{-1} of dry rhizospheric soil (Table 2) decreased throughout the experiments although numbers were consistently lower in mycorrhizal plants.

Shoot phosphorus content (% P in dry matter) was increased by the VA mycorrhizal fungus, but was unaffected by the pathogenic bacteria (Table 3). When *P. syringae* was inoculated in the absence of *G. mosseae* higher N and lower K content were observed.

Data from plants grown in a soil-sand mixture of 1:1 were similar to those presented.

DISCUSSION

P. syringae depressed the growth of non-mycorrhizal tomato plants, but growth of VA mycorrhizal plants was not affected. As with other plant pathogenic organisms (Dehne, 1982), the protection of plants from *P. syringae* was greater when pre-infected with the mycorrhizal fungus than when both microorganisms were inoculated simultaneously. This effect has been associated with an improved P nutrition of the plants by the VA endophyte (Davis

Table 2. *P. syringae* colony-forming units (cfu) of rhizosphere soil (g^{-1} dry wt of soil) of tomato plants grown in soil-sand mixture (1:9 v/v), inoculated or uninoculated with *G. mosseae*

Inoculation time	Treatments	$(10^2 \times \text{g}^{-1}$ dry rhizosphere soil)		
		Days after inoculation		
		5	20	35
<i>P. syringae</i> inoculated at the same time as <i>G. mosseae</i>	P	162	185	114
	M + P	124	74	53
<i>P. syringae</i> inoculated 3 weeks after <i>G. mosseae</i>	LSD 5%	50	31	21
	P	170	132	96
	M + P	119	65	43
	LSD 5%	55	42	18

Legend as for Table 1.

Table 3. Shoot N, P and K content (% dry matter) of tomato plants grown in soil-sand mixture (1:9 v/v) inoculated or uninoculated with *G. mosseae* in presence or absence of *P. syringae*

Inoculation time	Treatments	Content (% dry matter)		
		N	P	K
<i>P. syringae</i> inoculate at the same time as <i>G. mosseae</i>	C	1.03	0.06	2.83
	P	3.72	0.06	1.47
	M	1.08	0.08	2.82
	M + P	2.33	0.10	2.56
<i>P. syringae</i> inoculated 3 weeks after <i>G. mosseae</i>	LSD 5%	0.15	0.01	0.37
	C	1.01	0.05	2.52
	P	3.74	0.06	1.15
	M	1.09	0.08	2.52
	M + P	1.90	0.09	2.71
	LSD 5%	0.10	0.01	0.43

Legend as for Table 1.

and Menge, 1980). Enhanced nutritional status of the host plant cannot be excluded as a factor responsible for the induced resistance in the present experiments. However, the reduction in the *P. syringae* population was independent of the time interval between the inoculation of both microorganisms and of the nutrient concentration of soils. Thus it is still possible that the mechanisms of protection can be due not only to nutritional superiority associated with the infection by the VA endophyte but also to other factors connected with the mycorrhizal fungus. In this sense more research will be needed on the influence of VA mycorrhizas on pathogenic bacteria in order to determine whether the changes in host resistance are directly or indirectly related to improved host nutrition.

G. mosseae protected tomato plants against the pathogenic effect of *P. syringae* when both microorganisms were inoculated at the same time. This suggests the possibility of an interaction between the endomycorrhizal fungus and the pathogen in the extramatrical phase of the VA fungus. Similar mechanisms with other pathogens have been posited (Caron *et al.*, 1985).

This work emphasizes the necessity of further investigations on the possible production of inhibitory compounds by VA mycorrhizal fungi or their host and the ecological conditions that could enhance these advantageous effects.

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