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The Role of Vesicular-Arbuscular Mycorrhiza in Controlling Damping-off and Growth Reduction in Cucumber Caused by *Pythium ultimum*

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Abstract

Interactions between *Pythium ultimum* Trow and two strains of vesicular-arbuscular mycorrhizal (VAM) fungi (*Glomus* spp.) were studied with cucumber (*Cucumis sativus* L.). VAM inoculation before or simultaneous with the inoculation of the pathogen increased survival of the seedlings. Inoculation with *P. ultimum* 14 days after sowing did not kill the plants, but reduced the leaf area. This reduction was almost eliminated by one of the VAM isolates.

Introduction

Several studies have shown that root colonization by VAM fungi can decrease plant diseases caused by fungal root pathogens (Dehne 1982, Dehn and Dehne 1986, Rosendahl 1985, Krishna and Bagyaraj 1983). Rosendahl (1985) reported that only a well established VAM infection could reduce the damage caused by *Aphanomyces euteiches* Dreschl., but Krishna and Bagyaraj (1983) found that even the simultaneous addition of a mycorrhizal fungus and the pathogen *Sclerotium rolfsii* Curzi to peanut could reduce severity of disease. The pathogens investigated so far have been sublethal to the host plants, whereas protection of VAM against damping-off has not been reported.

The present study was designed to investigate the role of VAM for survival of cucumber seedlings after inoculation with the root pathogen *P. ultimum* and to study the influence of VAM on the reduction of plant growth caused by *P. ultimum*.

Materials and Methods

Pythium inoculum.

The isolate of *P. ultimum* (provided by H. Wolffhechel, Royal Veterinary and Agricultural University, Copenhagen) was isolated from a cucumber seedling with

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spontaneous damping-off. Inoculum consisting of oospores in vermiculite was prepared (Al-Hamdani et al., 1983) and dried in Petri dishes at room temperature. Four ml of inoculum was added to each plant, as this was found to cause maximum damping-off in a pilot test.

VAM fungi

Two strains of VAM fungi were used: *Glomus etunicatum* Becker and Gerd and *Glomus* sp. Inocula were produced on maize plants grown in calcinated montmorrillonite clay (Terra Green) for two months in a greenhouse, and consisted of spores, mycelia and infected roots.

Host plant

Cucumber seeds (cv. Aminex F.1.) were sown and inoculated with VAM by placing 2 ml of inoculum below the seeds. The plants were grown in speedling trays (75 ml vermiculite/pot; grade 2) and maintained in a glasshouse (20–25°C). Natural light was supplemented by high pressure sodium vapor lamps (30 watt/m^2) 24 hours/day.

Experimental design

There were six plants in each treatment, and all the plants were randomly placed. Two experiments were performed:

Experiment 1 included the following treatments: (a) non-VAM plants ± *P. ultimum* inoculation; (b) inoculation with either *G. etunicatum* or *G.*sp. at sowing time ± *P. ultimum* inoculation. The plants were inoculated with *P. ultimum* at 0, 2, 4, 9 and 11 days after sowing. Plants were harvested after 20 days and damping-off was recorded. Dry weight (70°C for 24 hr.) of each plant was recorded. The root systems were cleared in 10% KOH and stained in 0.05% tryphan blue in lactoglycerol. The percentage root length infected with VAM was determined; the roots were also examined for presence of *P. ultimum* oospores. Leaf areas were determined from measurement of the leaf diameters.

Experiment 2. The experimental design was as described in Exp. 1, but the plants were inoculated with *P. ultimum* only at 14 days after sowing. Leaf area was determined after 25 and 40 days. The plants were harvested after 40 days. The roots were treated as described for Exp. 1.

Results

In Exp. 1, inoculation with VAM decreased damping-off caused by *P. ultimum*, and the incidence of damping-off declined with increasing plant age before inoculation with *P. ultimum*. Full protective effect by *G.*sp. and *G. etunicatum* was found at four

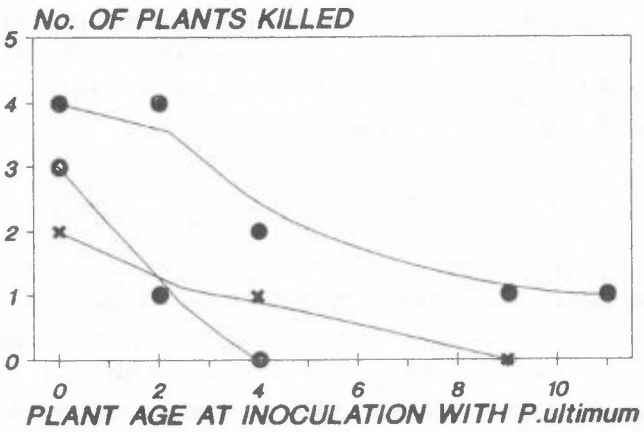

Figure 1. Influence of the time of inoculation with *P. ultimum* on the incidence of damping-off of cucumber plants. ●:non-VAM; o:inoculated with G.sp; x:inoculated with *G. etunicatum*.

and nine days, respectively (Fig. 1). The surviving plants showed no significant difference in dry weight or in leaf area compared to the controls. Oospores were found in roots of all plants inoculated with *P. ultimum*, but no attempts were made to quantify them. The root length infected with either of the two VAM isolates did not exceed five percent.

In Exp. 2, none of the plants inoculated with *P. ultimum* 14 days after sowing died, although roots were heavily infected with oospores found in them. The pathogen reduced the leaf area (Table 1) when measured after 25 days, but this reduction was less pronounced after 40 days. *G. etunicatum* did not promote plant growth when

Table 1. The influence of inoculation with VAM and *P. ultimum* (P.u.) on the leaf area (cm^2) of cucumber plants at day 25 and 40 after sowing and VAM inoculation.

	Leaf area (cm^2)	
Treatment	Day 25	Day 40
Non-VAM, non-P.u.	45.0 B	112.9 Y
Non-VAM + P.u.	27.5 C	93.9 YZ
G.sp.	63.4 A	160.2 X
G.sp. + P.u.	27.9 C	66.8 Z
G. etunicatum	48.1 B	116.1 Y
G. etunicatum + P.u.	37.5 BC	88.6 YZ

Means followed by the same letters are not significantly ($P > 0.05$) different as determined by Duncan's Multiple Range test.

measured after 25 and 40 days, but prevented or eliminated the growth reduction caused by *P. ultimum*. Plants inoculated with *G.*sp. produced the largest leaf area, but did not overcome the negative effect caused by *P. ultimum*. The percent root length infected with VAM ranged from 12 to 30.

Discussion

The protection of VAM against root pathogens has previously been demonstrated, mainly with sub-lethal diseases (Dehne 1982; Dehn and Dehne 1986). In the present experiment (Exp. 1), this protective effect by VAM inoculation was found when the plants were inoculated with the pathogen simultaneously with VAM inoculation. This protective effect is unexpected, as the VAM infection is not establishd when the pathogen is introduced. The protective mechanism remain unknown, but could be the result of an induction of inhibitory metabolites in the host plant as a response to VAM initiation. However, the pathogen could also be inhibited directly by the VAM fungus by compounds liberated from the VAM spores or mycelium. In Exp. 2 (Table 1) with pre-established VAM, *P. ultimum* caused no plant death, but significantly decreased leaf area at 25 days after sowing and VAM inoculation. The decrease was almost eliminated after 40 days. This indicates that the plants could overcome the growth reducing effect caused by *P. ultimum*.

The growth promoting effect of *G.* sp. seen in Exp. 2 was not correlated with the ability to reduce the negative effect caused by *P. ultimum*. This illustrates the relevance of including disease resistance, and not only growth enhancement caused by an increase in phosphorous uptake, when screening for efficient VAM isolates for commercial use in greenhouse plants.

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