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Short Communication

Control of root zone pH is not effective in preventing *Pythium aphanidermatum* disease in cucumber

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Abstract

Pythium aphanidermatum is the most wide-spread root pathogen in greenhouse cucumber. Although environmental conditions are known to affect the disease severity and many *in vitro* studies have documented these effects, only few *in vivo* experiments have been conducted, mainly focusing on the effect of temperature. In the present study, the impact of the pH in the root environment was investigated. Mature cucumber plants were grown in aerated nutrient solution at pH values of 4.0, 5.5 and 7.0, and inoculated with *P. aphanidermatum*. Experiments were carried out at temperatures of 20, 25 and 30°C. No clear effect of root zone pH on mycelium density in the roots measured by means of an indirect ELISA, and growth reduction of inoculated cucumber plants could be observed, probably due to different responses of the mycelium, oospores and zoospores of *P. aphanidermatum* to pH.

Key words: Hydroponic, indirect ELISA, mycelium, nutrient solution, plant growth

1 Introduction

Pythium aphanidermatum is the most wide-spread and most dangerous pathogen in greenhouse-grown cucumber (Al-Sa'di et al. 2008, Menzies et al. 1996, Moulin et al. 1994). Environmental conditions are known to affect the population dynamics of the pathogen. However, although the biology and ecology of *P. aphanidermatum* has been widely studied, mainly *in vitro* (e.g. Martin & Loper 1999, Middleton 1943), knowledge of the environmental effects *in vivo* is limited to that of the temperature (Kyuchukova et al. 2006, Panova et al. 2004, Raftoyannis & Dick 2002).

The effect of the pH on *P. aphanidermatum* is investigated in a number of *in vitro* studies. There is consensus on the behaviour of oospores: the germination rate increases with pH and is optimum at around pH 7 (Mondal & Hyakumachi 2000, Ruben et al. 1980, Adams 1971). However, reports on mycelium growth are inconsistent. Griffin (1958) reported a positive correlation of mycelium growth and pH *in vitro* for *Pythium ultimum* and Abdelzaher et al. (1997) described the same for *P. aphanidermatum* on cornmeal agar. By contrast, Panova et al. (2004) found the best *P. aphanidermatum*-mycelium growth on carrot agar at pH 5.0, while Holmes et al. (1998) identified the large range from pH 4.5 to pH 7 in compost to be suitable for the fast growth of *P. ultimum*.

In hydroponic greenhouse production, the pH in the root environment may be controlled automatically in a wide range. For this reason, in this study the effect of the pH on the disease severity of *P. aphanidermatum* in cucumber was investigated *in vivo* on mature plants, with the aim of possibly slowing down the epidemiology.

2 Materials and methods

P. aphanidermatum isolate BBA 70417 was routinely maintained on carrot agar plates. Oospores for plant inoculation and mycelium for standardisation of the indirect ELISA were produced in carrot broth, according to Elad & Chet (1987). The mycelium density in cucumber roots was measured using an indirect ELISA, as described by Kyuchukova et al. (2006).

Five experiments with cucumber (*Cucumus sativus* cv. Corona) were carried out in growth chambers (2.5 m × 4.0 m). Plants were cultivated in 12 l containers in aerated nutrient solution at an air temperature of 25°C, a relative humidity of 80% and a CO_2 concentration of 350 μmol mol^{-1} on a 14:10 h light:dark cycle at a photosynthetic active radiation (PAR) of 300 μmol m^{-2} s^{-1} (high pressure sodium lamps, Philips SONT-T 400W). The composition of the nutrient solution was based on recommendations for cucumber production in hydroponic growing systems (De Kreij et al. 1997) with pH 5.5. The volumes of nutrient solution taken up by the plants were replaced three times per week.

When the tenth true leaf had unfolded, air temperature in the chamber was gradually changed over two days to 20°C (experiments I and IV) and 30°C (experiment III), while in experiments II and V it was kept at 25°C. At the same time, the pH of the nutrient solution was varied by manually adding nitric acid or lime potash, with an aim of achieving pH values of 4.0, 5.5 and 7.0. This adjustment was repeated every day. *P. aphanidermatum* inoculum was then added to some of the containers, resulting in final densities of 10^5 (high, experiments I-III) and 10^4 (moderate, experiments IV-V) oospores l^{-1}. Each treatment included three replications and the experimental design was balanced in experiments I-III, while in experiments IV-V only the pH 5.5 served as non-inoculated control with four replications. Replications were randomly distributed in three or four blocks, respectively.

Plants were topped at 20 leaves. All side shoots, with the exception of the upper two, were removed. Ripe fruits were harvested. The experiments were terminated after having harvested all stem fruits, which means at the same phenological stage but after different growing times. Parts of the roots were stored at −20°C, and their *Pythium* mycelium density was later estimated using ELISA. Root samples of the non-inoculated plants were checked for possible infection by plating on carrot agar plates. Plants were harvested, and the masses of the roots, stem, leaves and fruits were recorded. Dry matter contents were measured after drying samples at 80°C for two

days. In the event of the sudden death of a plant, it was also immediately examined as described above.

Data were evaluated by analysis of variance using Fisher's F-test, followed by Tuckey's T-test at significance level $\alpha = 0.05$. The symbol p denotes the probability level of the corresponding test.

3 Results and discussion

Temperature significantly affected the speed of the phenological development of the plants. Thus, cucumbers were cultivated for 31 days after pathogen inoculation at 20°C, for 21 days at 25°C and for 17 days at 30°C, respectively. In one exceptional case, experiment II at 25°C was terminated after 15 days because all inoculated plants had already faded away due to lower stem rot.

Temperature had a significant effect on the yield, shoot and root growth of the non-inoculated plants as a result of different integrals of PAR absorbed by the plants over the different lengths of the experimental periods ($p < 0.001$). However, no effects of pH on plant growth characteristics ($0.43 < p < 0.80$) and no interactions of pH and temperature ($0.41 < p < 0.98$) could be observed in experiments I-III. In the following, therefore, the non-inoculated pH treatments in experiments I-III were pooled.

A tendency towards a lower pathogen density in the roots at low pH was observed at the high inoculum density (Fig. 1). Thus, mycelium density at pH 4.0 differed significantly from those at pH 5.5 and 7.0 when experiments I-III were evaluated together. However, this trend was not repeated at the moderate inoculum density (Fig. 1).

The lowest mycelium density in the roots at pH 4 and at high inoculum density resulted in the largest root mass among the pathogen-inoculated plants. Surprisingly, root growth was also maximum at pH 4.0 at the moderate inoculum density, despite the different figure in the mycelium density (Fig. 1, Table 1). Shoot growth and yield generally followed root growth, with the exception of experiment II in which all inoculated plants faded away due to lower stem rot. Here, unexpectedly, all plants with the lowest root mycelium density at pH 4 died just seven days after pathogen inoculation, which was significantly earlier than the 13 and 10 days after inoculation at pH 5.5 and 7, respectively (data not shown).

The impact of the root zone pH is obviously very complex, due to the various organs of the pathogen involved. Thus, the relatively lower mycelium densities of *P. aphanidermatum* in the cucumber roots and the lower root growth reduction at the high inoculum density at pH 4.0 could have been caused by the repeated *in vitro* measured low germination rates of oospores at low pH (Mondal & Hyakumachi 2000, Ruben et al. 1980, Adams 1971). This may have resulted in a slower propagation of the zoospores and, finally, in a lower primary infection rate. Moreover, indirect effects via antagonists could also have been involved (Ongena et al. 1999). The growing systems were not sterile and thus the antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG) producing *Pseudomonas* spp. may have been present. De Souza et al. (2003) reported a high sensitivity of the zoospores to 2,4-DAPG and an increasing activity of 2,4-DAPG against *Pythium ultimum* with decreasing pH, which may have contributed to a reduction in the disease incidence at pH 4 (Fig. 1). This phenomenon was not repeated at moderate inoculum density. It could be that the mycelium growth in the roots was more important here than the primary infection. As stated above, there is no consensus in the literature concerning the pH effect on mycelium growth *in vitro*. Moreover, the evaluation of a pH effect on mycelium growth *in vivo* is more difficult because the mycelium grows in hydroponics inside the roots, where the pH ranges between 5.6 and 5.9 in the xylem and between 7 and 8 in the phloem (Marschner 1995), and thus differs considerably from that in the root environment. It is likely that the pH effect on the epidemiology is indirect via changes in ion uptake, the defence mechanism of the plants and the population dynamics of beneficial micro-organisms (Bar-Yosef et al. 2008). Thus, the dry matter content of the cucumber roots significantly increased on average over all five experiments from 0.0360 g g^{-1} in the non-inoculated plants to 0.0447 g g^{-1} in the inoculated plants at pH 4.0, to 0.0505 g g^{-1} at pH 5.5 and to 0.0522 g g^{-1} at pH 7.0, which may indicate a weaker adaptation to the disease at lower pH and may partly explain the greater sensitivity to lower stem rot. This, however, requires further investigation. Some micro-organisms reduce mycelium growth in the roots (e.g. Höflich & Ruppel 1994); however it is questionable whether these processes depend on the pH in the root environment.

Concluding, the control of the pH is less suitable for preventing *P. aphanidermatum* disease in cucumber.

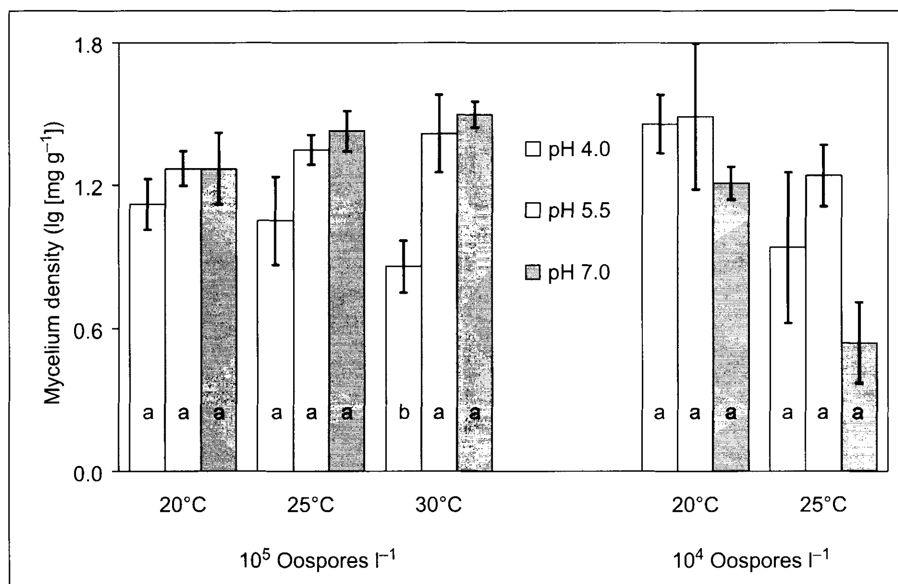

Fig. 1: Density of *P. aphanidermatum* mycelium in cucumber roots depending on root zone pH at different temperatures and pathogen inoculum densities. Bars represent standard error of mean. Columns with the same letter are not significantly different (Tukey's T-test at significance level $\alpha = 0.05$, separately for each experiment).

Table 1: Plant characteristics affected by an inoculation with *P. aphanidermatum* and the pH in the root environment. Prior to the pooled analysis (average), all data were divided by the mean of the corresponding non-inoculated treatment. Values in one line followed by the same letter are not significantly different (Tukey's T-test at significance level α = 0.05).

Inoculum density	Temperature	Plant characteristic	Control	*P. aphanidermatum*		
			pH 5.5	pH 4.0	pH 5.5	pH 7.0
10^5 Oospores l^{-1}	20°C	Root mass (g)	839 a	320 b	253 b	248 b
		Shoot dry matter (g)	266 a	186 b	185 b	185 b
		Yield (g)	3001 a	2264 ab	2267 ab	1961 b
	25°C	Root mass (g)	313 a	119 b	101 b	108 b
		Shoot dry matter (g)	124 a	49 c	80 b	60 bc
		Yield (g)	1498 a	16 c	734 b	197 bc
	30°C	Root mass (g)	182 a	157 a	86 b	80 b
		Shoot dry matter (g)	125 a	91 ab	66 b	57 b
		Yield (g)	1716 a	922 b	528 b	359 b
	Average	Root mass (%)	100 a	54 b	37 c	36 c
		Shoot dry matter (%)	100 a	67 b	62 b	54 b
		Yield (%)	100 a	43 b	52 b	33 b
10^4 Oospores l^{-1}	20°C	Root mass (g)	583 a	396 ab	291 ab	237 b
		Shoot dry matter (g)	229 a	190 a	182 a	189 a
		Yield (g)	2646 a	2474 a	1821 a	2066 a
	25°C	Root mass (g)	226 a	219 a	116 b	162 b
		Shoot dry matter (g)	139 a	127 a	92 b	126 a
		Yield (g)	1964 a	1726 a	940 b	1755 a
	Average	Root mass (%)	100 a	83 a	51 b	56 b
		Shoot dry matter (%)	100 a	87 a	73 b	86 ab
		Yield (%)	100 a	91 a	53 b	84 a

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