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Influence of the endomycorrhizal fungus *Glomus mosseae* on the development of peanut pod rot disease in Egypt

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Abstract The interaction between the arbuscular mycorrhizal fungus *Glomus mosseae* and the two pod rot pathogens *Fusarium solani* and *Rhizoctonia solani* and subsequent effects on growth and yield of peanut (*Arachis hypogaea* L.) plants were investigated in a greenhouse over a 5-month period. At plant maturity, inoculation with *F. solani* and/or *R. solani* significantly reduced shoot and root dry weights, pegs and pod number and seed weight of peanut plants. In contrast, the growth response and biomass of peanut plants inoculated with *G. mosseae* was significantly higher than that of non-mycorrhizal plants, both in the presence and absence of the pathogens. Plants inoculated with *G. mosseae* had a lower incidence of root rot, decayed pods, and death than non-mycorrhizal ones. The pathogens either alone or in combination reduced root colonization by the mycorrhizal fungus. Propagule numbers of each pathogen isolated from pod shell, seed, carpophore, lower stem and root were significantly lower in mycorrhizal plants than in the non-mycorrhizal plants. Thus, *G. mosseae* protected peanut plants from infection by pod rot fungal pathogens.

Key words *Arachis hypogaea* · Bioprotection · *Fusarium solani* · Pod rot disease · *Rhizoctonia solani* · *Glomus mosseae*

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Introduction

Pod rot disease of peanut is a serious problem in Egypt and worldwide. Among pathogens that threaten peanut production are *Rhizoctonia solani* (Kühn) and *Fusarium solani* (Mart.), which cause seed decay, seedling damping off, peg, pod and root rot (Garcia and Mitchell 1975; Walker and Csinos 1980; Porter et al. 1990; Davis et al. 1996; Saleh 1997; Kemerait and Kucharek 1998). All Egyptian commercial cultivars of peanut are susceptible to pod rot pathogens (Abu-Arkoub 1973; El-Wakil et al. 1984; Saleh 1997).

In Egypt, the peanut crop is usually grown in low-fertility sandy soil, which favors mycorrhizal development. Mycorrhizas are important components of intensively managed peanut plants because mycorrhizal colonization increases growth and yield, especially in soils with low fertility where the symbiont increases efficiency of nutrient absorption by plant roots (Azcon-Aguilar and Barea 1978; Hwang 1988; Johansen 1995; Guo et al. 1996; Abdel-Fattah 1997; Li et al. 1997; Tee Boon et al. 1997). Arbuscular mycorrhizal (AM) fungi also play an important role in the life of plants by reducing the susceptibility or increasing the tolerance of the host roots to soil-borne pathogens (Dehne and Schonbeck 1975; Schonbeck and Dehne 1977; Caron 1989; Jalali et al. 1990; Abdel-Fattah and Mankarios 1995; Liu 1995; Liu and Liu 1995; Baby and Manibhushanrao 1996; Cordier et al. 1996; Torres-Barragan et al. 1996; Azcon-Aguilar and Barea 1997; Bodker et al. 1998; Yao et al. 1998; Slezack et al. 1999).

Zambolim and Schenck (1983) showed that damage of soybean plants by *R. solani* and/or *F. solani* was reduced by prior root colonization by *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe. Tomato wilt caused by *F. oxysporum* was also reduced by mycorrhizal colonization (Dehne and Schonbeck 1975). In addition, Hwang et al. (1992) showed that AM fungi can reduce *Verticillium* and *Fusarium* wilt

incidence in alfalfa as well as pathogen propagule numbers in the soil. Torres-Barragan et al. 1996 reported that mycorrhizal fungi delayed onion white rot symptoms caused by *Sclerotinia cepivorum* by 2 weeks and provided a significant protection against the disease for 11 weeks after onion transplanting. Mycorrhizal plants showed an increase in yield of 22 % regardless of the presence of the white rot pathogen. Bodker et al. (1998) reported that *G. intraradices* (Schenck and Smith) reduced the development of root rot disease caused by *Aphanomyces euteiches* Drechs. in pea (*Pisum sativum* L.). In contrast, plant disease was not influenced or was increased by root colonization with AM fungi in some studies (Ross 1972; Davis 1980; Reddy et al. 1989). It is difficult to generalize on the effect of mycorrhiza on plant diseases because the interaction between arbuscular mycorrhizas and root-infecting fungi varies with the species of mycorrhizal fungi and the plant cultivar (Schonbeck 1979; Liu and Liu 1995).

The objectives of this present study were to investigate the interactions between the AM fungus *G. mosseae* and the pod rot pathogens *F. solani* and *R. solani* and the subsequent effects on growth and yield of peanut plants at different stages.

Materials and methods

Experimental design

The experiment was carried out in a completely randomized eight-block design with 30 replicates for each treatment. The eight treatments were as follows: control (sterile soil); soil with mycorrhiza only (M); soil infested with *F. solani* only (P1); soil with M+P1; soil infested with *R. solani* only (P2); soil with M+P2; soil infested with P1+P2; soil with M+P1+P2.

Production of pod rot pathogens and vesicular-arbuscular mycorrhizal inoculum

Fusarium solani (Mart.) and *Rhizoctonia solani* (Kühn) were isolated originally from infected peanut plants collected from fields in El-Ismailia province, Egypt. Pure inoculum of each fungus was produced in 250-ml conical flasks containing an autoclaved medium composed of crushed peanut seed and pod shells. The medium was inoculated with a 9-mm-diameter mycelial disk of *F. solani* or *R. solani*. Inoculated flasks were incubated without shaking for 7 days at 22 ± 2 °C under 12 h of fluorescent light per day. Cultures of each fungus were diluted with sterilized sand (1 : 2, v/v) as carrier. Each pot received 10 g of inoculum mix containing 2×10^4 conidia for *F. solani* or ca. 50 sclerotia plus mycelium for *R. solani*. Uninoculated control pots received 10 g of sterilized inoculum mixture.

Glomus mosseae (Nicol. and Gerd.) Gerd. and Trappe was isolated from the rhizosphere of peanut plants and maintained on roots of leek (*Allium porrum* L.) seedlings grown in a steam-sterilized mixture of sand and loam soil (1 : 1, v/v) in plastic pots in a growth chamber (16-h photoperiod, relative humidity 80 %, air temperature 22 ± 2 °C, light intensity 400 $\mu E.m^{-2}s^{-1}$). Two months after inoculation, leek roots were collected, chopped and mixed into the sand and loam soil mix. The mixture of soil, chlamydospores and mycorrhizal-colonized root segments was stored at 4 °C for 3 days until use.

Plant and growth conditions

Seeds of a peanut cultivar (*Arachis hypogaea* L cv. Giza 5) susceptible to pod rot disease were surface sterilized with 0.5 % sodium hypochlorite for 5 min, rinsed three times in sterilized distilled water, then left to germinate for 3–4 days at 28 ± 2 °C rolled in moist, sterilized filter papers. Germinated seedlings with uniform radicles were planted (one seedling per pot) in 30-cm-diameter pots containing 3 kg of autoclaved sand and loam soil mixture (1 : 1, v/v). Soil characteristics were pH (water) 7.8, 502 mg/kg total phosphorus, 18 mg/kg available P (Olsen), 13 mg/kg available nitrogen, 0.11 % total nitrogen and 1.6 % $CaCO_3$. Half of the young plants received mycorrhizal inoculum consisting of 0.5 g chopped leek roots colonized by *G. mosseae* (stock pot culture, M = 72 %) and 30 sporocarps per pot. The mycorrhizal inoculum was placed 5 cm below the soil surface at planting. Filtered washings of the inoculum and autoclaved mycorrhizal leek roots were added to non-mycorrhizal treatments to provide the same microflora without *G. mosseae*. All seeds were inoculated with *Rhizobium* obtained from the Microbiology Laboratory, Sakha Agriculture Research Station, Egypt. After 1 month, the upper part of the root system of part of the plants was uncovered and the rhizosphere of each plant was inoculated with the corresponding pathogen inoculum prepared as described above, then immediately covered with soil. All pots were arranged randomly in the greenhouse under natural conditions of temperature, daylight, light intensity and day length during the summer season. The pots were irrigated regularly to near field capacity with tap water. After 4 weeks, all plants received K_2SO_4 at 32 mg per pot.

Evaluation of plant growth and mycorrhizal root colonization

Ten replicates of the plants from each treatment were harvested at 45 days (vegetative stage), 90 days (flowering stage), and 150 days (maturity stage) after planting. Immediately after each harvest, the root systems were washed carefully with tap water to remove adhering soil particles. A weighed sample of the root system was cut into 0.5- to 1-cm segments for estimation of mycorrhizal colonization after clearing and staining with trypan blue (Phillips and Hayman 1970). The frequency of mycorrhizal colonization (F %), intensity of root cortex colonization (M %) and arbuscule frequency (A %) were determined microscopically as described by Trouvelot et al. (1986). Dry weights of shoots and roots were determined after drying at 70 °C for 24 h. Growth parameters including number of branches, pegs and pods per plant were estimated during different growth stages. In addition, the weights of 100 pods and 100 seeds were evaluated at maturity.

Disease incidence and severity

For disease incidence, the frequencies with which *F. solani* or *R. solani* could be isolated from different plant organs were determined *in vitro* at the last harvest. Ten plants of each treatment were taken randomly (with or without symptoms) and separated into lower stem, root, carpophore, peg, pod shell and seed. Three replicates of 10 pieces of each organ were surface sterilized with 0.5 % sodium hypochlorite for 2 min, rinsed three times in sterile distilled water and placed on PDA plates (5 pieces per plate). All plates were incubated at 25 °C for 5–7 days with 12 h of alternating fluorescent light and darkness. The percentage disease incidence for each fungus was assessed from the proportion of infected pieces.

Disease severity in individual plants was rated on a scale of 0–5 as described by Hwang et al. (1992), where 0 = no disease symptoms, 1 = < 25 % disease symptoms, 2 = 25–50 % disease symptoms, 3 = 50–70 % disease symptoms, 4 = 75–100 disease symptoms, 5 = dead plants. The individual ratings were converted to mean percent infection using (sum of individual plant rating values × 100) / (5 × number of plants diseased).

Data were subjected to ANOVA using Costat 1990 (Cottort Software, Berkeley, Calif.) and means were compared using Duncan's multiple range test.

Results

Growth responses

At all growth stages, shoot and root dry weights of peanut plants inoculated with *G. mosseae* were significantly higher than those of non-inoculated control plants or plants infested with the pathogens either singly or in combination (Table 1). On the other hand, peanut plants infected with *F. solani* and / or *R. solani* showed significantly lower dry weights, number of branches and pegs number than the non-inoculated control plants. The reductions were very pronounced in the case of combined pathogen treatments.

At vegetative and flowering stages, there were no significant differences between mycorrhizal and non-mycorrhizal plants for number of branches (Table 1). However, interactions between the mycorrhizal fungus and the pod rot pathogens had significant effects on shoot dry weight. *G. mosseae* inoculation of peanut plants prior to soil infestation with *F. solani* or *R. solani*, singly or in combination, significantly increased shoot and root dry weights, number of branches and pegs number compared with pathogen treatments alone.

Yield production

At the final harvest, pod and seed yield of peanut plants infected with *F. solani* and/or *R. solani* were significantly lower than those of non-inoculated control plants (Table 1). Peanut plants colonized with *G. mosseae* alone showed a significant increase in pod number, pod weight and seed biomass over non-mycorrhizal plants of other treatments, irrespective of the presence or absence of the pathogens. In the treatments combining pathogens and the mycorrhizal fungus, seed yield was significantly higher than with pathogens alone (Table 1).

Mycorrhizal root colonization

In general, the frequencies of root colonization (F %), intensity of root cortex colonization (M %), and arbuscule development (A %) by *G. mosseae* were affected by the pod rot pathogens at different growth stages.

Table 1 Effect of *Glomus mosseae* on growth responses and yield of peanut plants inoculated with *Fusarium solani* and/or *Rhizoctonia solani* at different growth stages. (*M* Mycorrhiza *Glomus mosseae, P1 Fusarium solani P2 Rhizoctonia solani*). For each growth stage, means in each column followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range

Growth stage	Treatment	Growth parameters				Yield biomass		
		Shoot dry wt. (g)	Root dry wt. (g)	No. of branches	No. of pegs	Pods per plant	100 pods wt. (g)	100 seeds wt. (g)
Vegetative	Control	2.31 b	0.23 c	3.0 a				
	M	2.74 a	0.28 a	3.0 a				
	P1	1.59 d	0.16 e	2.3 b				
	M+P1	1.79 cd	0.26 b	2.3 b				
	P2	1.29 e	0.15 f	1.7 c				
	M+P2	2.14 b	0.14 g	2.3 b				
	P1+P2	1.22 e	0.11 h	1.3 c				
	M+P1+P2	1.85 c	0.19 d	2.3 b				
	LSD(0.05)	0.22	0.01	0.47				
Flowering	Control	6.48 b	0.43 bc	5.0 a	5.3 a			
	M	8.18 a	0.54 a	5.0 a	4.7 ab			
	P1	3.75 e	0.27 de	4.0 ab	2.7 cd			
	M+P1	6.48 b	0.44 b	4.0 ab	3.0 c			
	P2	3.49 f	0.29 de	4.3 a	1.6 e			
	M+P2	5.66 c	0.33 cd	4.7 a	4.0 b			
	P1+P2	2.95 g	0.22 e	2.7 c	1.3 e			
	M+P1+P2	4.15 d	0.30 de	3.0 bc	2.0 de			
	LSD(0.05)	0.04	0.09	1.20	0.70			
Maturity	Control	9.28 b	1.12 e	6.3 b	6.1 b	9.7 c	191.43 b	64.50 b
	M	13.02 a	1.91 a	8.3 a	9.3 a	12.3 a	232.47 a	72.45 a
	P1	6.66 g	0.85 f	5.0 c	4.0 d	8.0 d	143.90 e	45.92 d
	M+P1	8.79 d	1.18 d	6.5 b	5.7 bc	10.6 b	189.22 b	65.96 b
	P2	6.89 f	1.22 c	5.0 c	5.0 c	7.0 e	124.76 f	39.76 e
	M+P2	9.08 c	1.13 e	6.7 b	6.3 b	8.7 d	189.92 b	60.54 c
	P1+P2	5.75h	0.88 f	5.0 c	3.7 d	5.3 f	149.10 d	38.42 e
	M+P1+P2	7.97 e	1.53 b	5.3 c	5.7 bc	8.7 d	184.14 c	63.49 b
	LSD(0.05)	0.04	0.34	0.73	0.97	0.75	2.92	2.71

Table 2 Influence of *Fusarium solani* and/or *Rhizoctonia solani* on the mycorrhizal colonization of peanut plants grown in sterilized soil. Values represent means of 40 root segments for each parameter. Means in each row for each stage followed by the same letter are not signifcantly different at $P = 0.05$ according to Duncan's multiple range. *A* % Arbuscular frequency in roots, *F* % root colonization, *M* % cortex colonization, *M* mycorrhiza *Glomus mosseae*, *P1 Fusarium solani*, *P2 Rhizoctonia solani*)

Growth stage	Mycorrhizal colonization (%)	Treatments								
		Control	M	P1	M+P1	P2	M+P2	P1+P2	M+P1+P2	LSD (0.05)
Vegetative	F %	0 d	43.0 a	0 d	40.0 ab	0 d	38.0 bc	0 d	35.0 c	3.4
	M %	0 b	32.5 a	0 b	32.1 a	0 b	31.2 a	0 b	30.8 a	2.1
	A %	0 c	31.5 a	0 c	30.0 ab	0 c	29.0 b	0 c	29.2 b	1.8
Flowering	F %	0 e	64.7 a	0 e	59.0 b	0 e	52.0 b	0 e	44.0 d	3.1
	M %	0 d	49.2 a	0 d	45.0 a	0 d	38.0 b	0 d	30.8 c	4.4
	A %	0 e	47.3 a	0 e	41.6 b	0 e	37.0 c	0 e	28.1 d	1.4
Maturity	F %	0 e	80.3 a	0 e	68.0 b	0 e	59.9 c	0 e	50.0 d	1.5
	M %	0 e	57.5 a	0 e	48.0 b	0 e	41.3 c	0 e	33.3 d	1.5
	A %	0 d	51.3 a	0 d	42.7 b	0 d	28.4 c	0 d	25.2 c	3.5

The frequencies of root segments and root length colonized by *G. mosseae* for plants infected with *F. solani* and/or *R. solani* were significantly lower than those for plants inoculated with the AM fungus alone. Similarly, the percentage of root system with arbuscules declined significantly when plants were concomitantly inoculated with the AM fungus and one or both pathogens. The reduction in mycorrhizal colonization was greatest when plants were inoculated with both pathogens. Mycorrhizal root colonization was not observed in control plants or those inoculated only with pathogens.

Disease incidence

As the final harvest, the frequency with which *F. solani* or *R. solani* was isolated from different peanut organs was significantly higher in non-mycorrhizal than in mycorrhizal treatments (Fig. 1). The highest frequencies of *F. solani* or *R. solani* were found associated with the pod shell, followed by carpophore, peg, lower stem and root.

Disease severity

Visual examination of peanut plants showed the presence of pod and root rot symptoms in all pots infested with the fungal pathogens. *R. solani* was more aggressive than *F. solani*, as seen from the higher percentages of dead plants, decayed pods and root rot (Fig. 2). Only control plants and those inoculated with *G. mosseae* alone were free of disease symptoms throughout the experiment. Development of pod and root rot symptoms in peanut plants was affected by the presence of the mycorrhizal fungus (Fig. 2). Taken individually, each mycorrhizal treatment markedly attenuated symptoms of pod and root rot disease and consequently reduced disease severity. Non-mycorrhizal plants grown in soil infested with one or both pathogens had higher disease severity ratings than mycorrhizal plants inoculated with the pathogens.

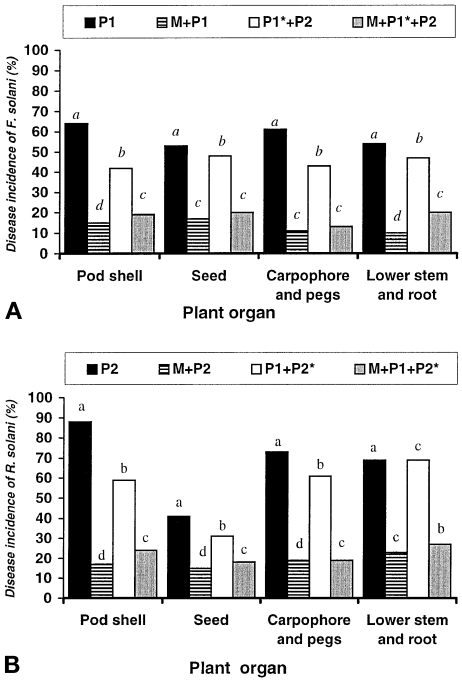

Fig. 1 Effect of *Glomus mosseae* on the percentage incidence of *Fusarium solani* (**A**) and *Rhizoctonia solani* (**B**) isolated from different peanut plant organs. Bars for each plant organ topped by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$) (*M* Mycorrhiza, *P1 Fusarium solani*, *P2 Rhizoctonia solani*)

Discussion

In the present study, infection by the pathogens *F. solani* and *R. solani* reduced growth and yield of peanut plants at all growth stages. Pre-inoculation of

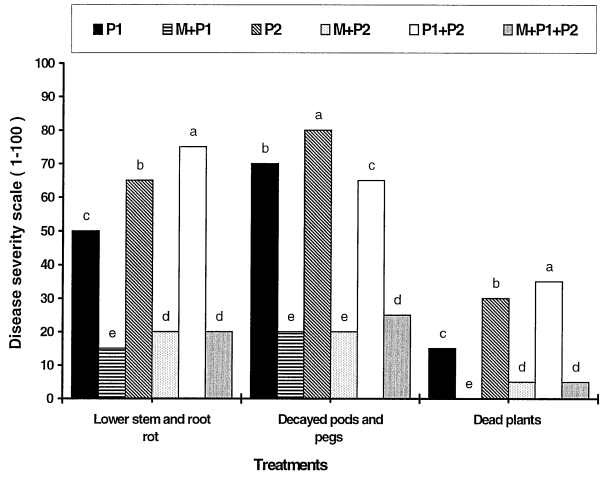

Fig. 2 Effect of *Glomus mosseae* on the disease severity of peanut pod rot caused by *Fusarium solani* and/or *Rhizoctonia solani*. Bars for each parameter topped by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$). Abbreviations as in Fig. 1

plants with *G. mosseae* attenuated the impact of the pathogens. Peanut plants inoculated with the AM fungus had a lower incidence of pod and root rot disease than non-mycorrhizal plants. These results confirm previous studies which indicated that root colonization by AM fungi can decrease the development of fungal root pathogens and the severity of the disease in their host plants (Schonbeck and Dehne 1977; Davis and Menge 1980; Zambolim and Schenck 1983; Caron et al. 1986; Hwang et al. 1992; Abdel-Fattah and Mankarios 1995; Carling et al. 1996; Liu and Liu 1995; Azcon-Aguilar and Barea 1997; Bodker et al. 1998; Yao et al. 1998). In contrast, Ross (1972) found that mycorrhizal infection increased root rot of soybean caused by *Phytophthora megasperma* var. *sojae*. Other *Phytophthora* species pathogenic to citrus and alfalfa were also unaffected by mycorrhizas (Davis et al. 1979; Davis and Menge 1981). The diversity of interactions between mycorrhiza, root invading pathogens and host plants suggests that the outcome depends on the host, the type and amount of inoculum of the pathogen, environmental conditions during the evaluation period and the mycorrhizal fungus involved (Davis and Menge 1980; Norman et al. 1996).

In the present study, development of pod and root rot symptoms in peanut was highly affected by the presence of an AM fungus. Lower disease severity, stimulation of plant growth, and reduction in frequencies of detected pathogens in the peanut organs were associated with the presence of the mycorrhizal fungus. Our observations agree with those of Hwang et al. (1992), who showed that alfalfa plants inoculated with AM fungi had a lower incidence of *Fusarium* wilt disease than non-mycorrhizal plants. Yao et al. (1998) also reported that the extent and severity of disease caused by *R. solani* in a highly susceptible potato cultivar was significantly reduced in AM plants. Gerdemann (1975) questioned whether altered disease resistance in mycorrhizal plants is attributable to improved plant nutrition or to other mechanisms. It appears that increased nutrient absorption by mycorrhizal roots alone cannot account for increased tolerance to pathogens (Hwang et al. 1992). The effect of AM fungi in plant protection may be via the production of phenolic or inhibitory compounds (Morandi 1990), altered root exudates (Bansal and Mukerji 1994) or changes in the microbial rhizosphere populations (Ames et al. 1984; Andrade et al. 1997). However, Bodker et al. (1998) and Cordier et al. (1998) reported that mycorrhizal fungi induce systemic resistance in pea and tomato plants infected with *Aphanomyces euteiches* and *Phytophthora parasitica*, respectively. Mycorrhizal plants have been found to contain higher amounts of amino acids such as arginine, and this amino acid when added to extracts of non-mycorrhizal roots inhibited chlamydospore formation by *Thielaviopsis basicola* (Baltruschat and Schonbeck 1975). Dehne and Schonbeck (1979) observed that mycorrhizal roots were more lignified and contained more polysaccharides than non-mycorrhizal roots, especially in the stele tissue. Cordier et al. (1998) showed that resistance to *P. parasitica* in mycorrhizal tomato root systems was related to induced defense responses in pathogen-colonized tissues. Furthermore, Abd-Alla (1997) reported that in mycorrhizal potato plants resistant to infection by *P. infestans* host epidermal cells underwent necrosis and pathogen development was restricted to epidermal cells with no penetration of host tissue cells by haustoria. Roots colonized by mycorrhizal fungi also exhibit greater chitosanase, chitinase and β-1,3-glucanase activities (Dumas-Gaudot et al. 1996; Kjoller and Rosendahl 1997; Pozo et al. 1998). These enzymes can be inhibitory to certain fungal pathogens (Dehne et al. 1978; Pozo et al. 1999).

In conclusion, this study suggests that the AM fungus *G. mosseae* can act as a bioprotective agent against *F. solani* and *R. solani*, the peanut pod and root rot disease pathogens. The results emphasize the need to investige further the mechanisms by which AM fungi induce resistance in their hosts and to better define the environmental conditions enhancing disease resistance.

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