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The effect of mycorrhizal fungi on the hatch of potato cyst nematodes

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Abstract

In the presence of potato plants cv. Golden Wonder, *Globodera pallida* exhibited delayed in-soil hatch compared to that of *G. rostochiensis*, with significantly fewer *G. pallida* second-stage juvenile nematodes hatching in the first two weeks, though the difference disappeared after four weeks. Inoculation of potato plants with arbuscular mycorrhizal fungi eliminated this delay in *G. pallida* hatch, so that the two potato cyst nematode (PCN) species exhibited similar in-soil hatch rates. When the corresponding *in vitro* hatching activities of root leachate from uninoculated and mycorrhiza-inoculated plants were compared, similar effects were revealed. *G. pallida* hatch in root leachates from uninoculated plants increased significantly from one-week-old to two-week-old plants, but this increase was not significant in the mycorrhizal-inoculated plants. When the in-soil experiment was repeated using the potato cyst nematode non-host plant strawberry, mycorrhizal inoculation induced no significant increase in *G. pallida* hatch. The results indicate that mycorrhizal inoculation of potato plants stimulates production of *G. pallida*-selective hatching chemicals, either hatching factors or hatching factor stimulants.

Keywords: Arbuscular mycorrhizal fungi (AMF); Potato cyst nematodes; *Globodera pallida*; *Globodera rostochiensis*; Microplants; Hatch

1. Introduction

Two species of potato cyst nematode (PCN) exist, *Globodera pallida* and *Globodera rostochiensis*, and they attack the roots of a small number of solanaceous species including potato, tomato and aubergine. Infestation results in reduced root growth (Trudgill, 1980). This leads to a reduction in the uptake of most minerals especially nitrogen, phosphorus and potassium (Trudgill et al., 1975). PCN-infested plants are often water stressed, and wilt and lose their leaves prematurely. In the field, PCN infestation symptoms appear as patches of sickly plants within the crop which enlarge as the infestation increases (Greco, 1988). PCN causes up to £300M sterling worth of damage to the potato crop in the EU each year.

PCN survives in the soil as spherical cysts, each containing up to 600 eggs. Each egg contains one unhatched second-stage juvenile nematode (J2). These juvenile nematodes hatch out in response to hatching factors (HFs) which are PCN host-specific chemicals leached from the roots (Jones et al., 1998). Once hatched, the J2 migrates to the host roots where it enters just behind the root tips or near the points of emergence of the lateral roots (Trudgill et al., 1996). The J2 then moves through the cortex, coming to settle in the inner cortex where the feeding action of the J2 stimulates development of a large feeding cell or syncytium. The J2 then moults twice, after which sexual

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differentiation occurs, and the males leave the roots. The females remain attached with the head buried in the root. Fertilization then occurs; the female nematode dies and her body tans, becoming a protective cyst for the eggs.

Factors that induce PCNs to hatch have been reported for potato root leachate (Devine et al., 1996). Results from this laboratory have shown the presence of multiple HFs (Devine et al., 1996), hatch inhibitors (HIs, root chemicals which inhibit HF-induced PCN hatch; Byrne et al., 1998) and hatching factor stimulants (HSs, which are hatch-neutral chemicals which stimulate HF-induced hatch; Byrne et al., 1998) in potato root leachate (PRL). Results have also shown that *G. pallida* and *G. rostochiensis* are selective in their response to individual HFs (Byrne, 1997). Devine et al. (1996) reported differences in the HF profiles of root leachates of sterile-grown and non-sterile-grown micropropagated potato plants ("microplants"). PRL collected from the non-sterile plants contained more HFs than PRL obtained from the plants grown under sterile conditions, suggesting that several of the HFs in conventionally-produced PRL may be microbial in origin.

Root-colonising micro-organisms include rhizosphere and rhizoplane bacteria and ecto- and endomycorrhizal fungi. The term mycorrhiza is used to describe the symbiotic association between plant roots and fungi. The vesicular-arbuscular endo-mycorrhizal (VAM) fungi belong to the order Glomales, and consist of six genera: *Gigaspora*, *Scutellospora* (family Gigasporaceae), *Glomus*, *Sclerocystis* (Glomaceae), *Acaulospora* and *Entrophospora* (Acaulosporaceae) (Morton and Benny, 1990). They are all obligate symbionts and do not form a root fungal sheath (unlike the Ectomycorrhiza). They penetrate the plant root tissue intercellularly. As the fungi develop in the host plant roots, they form several intracellular and extracellular structures that include extramatrical and intercellular hyphae, vesicles and arbuscules.

Research has indicated that mycorrhizal symbiosis can influence host development in terms of growth and yield, both positively and negatively. Benefits include reduced drought stress (Nelson, 1987), enhanced nutrient (particularly phosphorus) uptake (Gianinazzi-Pearson and Gianinazzi, 1983) and increased disease resistance (Dehne, 1982). Endomycorrhizal fungi have shown potential as biological control agents against pathogens of both strawberry (Vestberg, 1992; Mark and Cassells, 1996) and maize (Gerdemann, 1964). With VAM fungi and PCN occupying the same area of root tissue (Ingham, 1988), there is potential for biocontrol of PCN by mycorrhizal fungi. It has been demonstrated that VAM fungi can reduce infestation or damage by nematodes other than PCN (Hussey and Roncadori, 1982) , due primarily to stimulatory effects on root growth. More recent research showed that an increase in tolerance to root-lesion nematodes occurred when the host plant was inoculated with mycorrhizal fungi (Pinochet et al., 1996). Here the increase in tolerance seemed to be due to mycorrhiza-assisted nutrition rather than a direct suppressive effect of VAM on the nematode.

The reliance of PCN on hatching chemicals to complete their life cycle makes this step a potential target for biocontrol. Organisms which affect the host-mediated hatching process could be used in PCN control strategies. The objective of the study described here was to determine whether mycorrhizal colonisation affected the capacity of host and non-host plants to stimulate PCN hatch.

2. Materials and methods

2.1. Plant material

All potato plants used were microplants of cv. Golden Wonder, aseptically propagated via nodal culture (Curry and Cassells, 1998). Plant tissue stocks were cultured on half-strength M & S medium (Murashige and Skoog, 1962): 2.21 g l^{-1} Murashige and Skoog basal salts, 15 g l^{-1} sucrose, 100 μg l^{-1} kinetin, 200 μg l^{-1} gibberellic acid, 6 g l^{-1} agar, pH 5.8, and placed in a growthroom (photosynthetic photon flux rate 300 μmol m^{-2} s^{-1}, under a 16 h day at 22±2°C, relative humidity: day, 55%; night, 100%).

2.2. In vivo inoculation with VAM

Microplants were inoculated at the time of acclimatisation from culture vessels. Vaminoc (850±70 infective propagules per gram; MicroBio Division, Agricultural Genetics, Royston, Herts., UK) was placed (1 g) in each planting hole at the time of transfer to a peat-based potting compost (Bord na Mona,

Ireland; Duffy et al., 2000). Plants were misted daily for one week whilst in a growth chamber, followed by three weeks under standard glasshouse conditions of ambient temperature (minimum 16°C) and a 16 h photoperiod provided by daylight supplemented by high-pressure sodium vapour lamps (400 W). Plants were weaned to outdoor conditions for one week before experiments were set up. Uninoculated plants were produced in the same way, but without the inclusion of Vaminoc in the planting hole.

2.3. Determination of mycorrhizal colonisation

Samples of root material (5 g root sample per plant chopped into 1 cm pieces) were cleared and stained with 0.05% Trypan blue in lactic acid, and the percentage root length colonised (ten 1 cm sub-samples taken for percentage RLC) was determined using the grid line intersect method described by Giovannetti and Mosse (1980). Samples were taken at the four harvest dates (2, 4, 6 and 12 weeks after planting).

2.4. Potato cyst nematodes

Single-generation cysts of *G. rostochiensis* (pathotype Ro1) and *G. pallida* (Pa2/3) were used. Cysts were pre-soaked at 22°C on water-saturated discs of filter paper for one week prior to use.

2.5. Potato in-soil hatch experiment

Vaminoc-inoculated and -uninoculated microplants of potato cv. Golden Wonder were grown in 17.5 cm diameter pots of washed quartz sand supplemented with slow-release solid fertilizer (3 month Osmocote^{TM}; Grace Sierra, B.V. Herleen, the Netherlands) in the presence or absence of each PCN species. In order to aid their recovery at harvest, the cysts were placed in sealed cotton muslin sachets (3 cm × 3 cm), each containing 60 pre-soaked cysts of one PCN species and 1 g quartz sand, using a procedure modified from that of Brodie et al. (1976). Control pots (containing plants but no cysts, or containing cysts but no plants) were also set up. The sachets were placed in each pot at a depth of 1–2 cm below the root system at planting. Pots were incubated outdoors in the Carrigside experimental garden, National University of Ireland, Cork in a replicated randomised block design. The pots were covered with white oil cloth to reflect light from the pots to minimise temperature elevation. Six replicate pots of each of the eight samples were harvested at each of the four harvest dates (2, 4, 6 and 12 weeks after planting) making a total of 192 pots for the entire experiment. At each harvest the sachets were recovered and the percentage hatch determined.

To determine percentage hatch, three sub-samples of six cysts were recovered at random from each sachet and were immersed in 0.1% (w/v) aqueous Meldola's blue solution (Shepherd, 1962) for one week; samples had also been taken from the pre-soaked cysts prior to the experiment. The cysts were then washed and soaked in water overnight to remove the excess stain. Following this, the cysts were placed in 200 μl of water and gently opened mechanically to free the eggs into the water. Three sub-samples (each 20 μl) were taken after the egg suspension had been thoroughly mixed using a vortex mixer. For each sub-sample, the numbers of stained unhatched eggs (non-viable eggs), unstained unhatched eggs (viable) and hatched eggs (each representing a hatched juvenile) were counted and the percentage hatch of the viable eggs was obtained using the following formula (Byrne, 1997):

$$\%\ \text{Viable egg hatch} = \frac{\text{Number of hatched eggs} \times 100}{(\text{Number of hatched eggs} + \text{Viable full eggs})}$$

2.6. Strawberry in-soil hatch experiment

Strawberry microplants cv. Cambridge Favourite were inoculated with the commercial mycorrhizal inoculum Vaminoc as described by Mark and Cassells (1996). The strawberries were grown in 17.5 cm diameter pots of washed quartz sand containing sachets of cysts of individual PCN species (six replicates per treatment) as described above for the potato experiment. Differences from the potato experiment included the fact that there was only one harvest (after four weeks) and the experiment was conducted under glasshouse conditions (minimum temperature of 16°C, 16 h photoperiod, with natural daylight supplemented by 400 W sodium vapour lamps). The sachets

were recovered and the percentage hatch calculated as described previously.

2.7. In vitro hatch

Vaminoc-inoculated and -uninoculated potato microplants were grown in 17.5 cm diameter pots of washed quartz sand under glasshouse conditions (minimum temperature of 16°C, 16 h photoperiod, with natural daylight supplemented by 400 W sodium vapour lamps). Six replicate pots of each sample were set up for collection of root leachate each week for four weeks, starting one week after planting. Each pot was washed through with 1 l water at each collection date and the resulting leachate was collected. This procedure was conducted bi-weekly for each pot and the two leachates collected each week from a single pot were pooled. Following collection, the leachates were filtered (through 3 MM Whatman filter paper), to remove particulate material, and rotary evaporated to a volume of 10 ml (stock solution) and stored at 4°C until needed.

The *in vitro* hatching bioassay (Twomey et al., 1995) involved incubating 100 μl of the leachate samples with five pre-soaked cysts in microtitre plate wells (three sub-samples of each replicate leachate at each of four dilutions in a logarithmic series from the concentrated stock solution). Each sample was tested for activity towards both PCN species. Following three weeks incubation (in the dark at 22°C), the percentage hatch was determined by counting the number of hatched J2s (Twomey et al., 1995).

3. Results

3.1. Confirmation of mycorrhizal colonization

Successful mycorrhization of the potato microplants was confirmed in all Vaminoc-inoculated treatments. There was a general trend of increasing VAM colonisation of the potato root system with time, but this was only significant between week 2 and week 6 for the control (no PCN) plants (Table 1). The control plants exhibited significantly higher levels of VAM colonisation than did the PCN-infested plants at four weeks *(G. rostochiensis* only) and six weeks (both PCN species) (Table 1).

Table 1
Percentage root length colonisation for mycorrhizal plants in each of the three nematode treatments[a]

Treatment	Weeks after PCN inoculation			
	2	4	6	8
G. pallida	13.16a	12.33a	16.00abc	16.66abc
G. rostochiensis	14.66abc	13.33ab	15.66abc	17.00abc
No PCN	16.00abc	18.00bcd	22.00d	19.66cd

[a] Any two samples sharing a common letter were not significantly different ($p>0.05$) using the Tukey test; $n=6$ for each sample.

3.2. Effect of VAM on in-soil PCN hatch

At each harvest in the in-soil hatch experiment, the sachets were recovered and the percentage hatch of viable eggs calculated to determine whether the presence of mycorrhizal fungi on the roots affected the in-soil hatch of either PCN species. For both PCN species, hatch was significantly higher in the presence of potato plants (Fig. 1). Both spontaneous (in the absence of a potato plant) and induced hatch reached a maximum after four weeks, so the data from harvests at weeks 6–12 have been omitted from analysis and presentation. The spontaneous hatch of *G. rostochiensis* after four weeks was significantly greater than that of *G. pallida* (Fig. 1).

At week 2, the mean in-soil hatch of *G. pallida* under uninoculated plants (54.1%) was significantly lower than that of *G. rostochiensis* (81.9%; Fig. 1a). By week 4, the differences in hatch between the two PCN species had been eliminated (Fig. 1a). Vaminoc inoculation of the potato plants accelerated the hatch of *G. pallida* so that the delay in *G. pallida* hatch (relative to that of *G. rostochiensis*) induced after two weeks by uninoculated plants had been eliminated (Fig. 1b). No significant difference in percentage hatch of *G. rostochiensis* and *G. pallida* was observed in the presence of inoculated plants (Fig. 1b). There was no effect of mycorrhizal inoculation on *G. rostochiensis* hatch, indicating that the effect of the mycorrhizal fungi was *G. pallida*-specific.

3.3. Effect of VAM on in vitro PCN hatch

A parallel study was conducted on the in vitro hatching activity of root leachates from inoculated and uninoculated potato plants. It is apparent that the

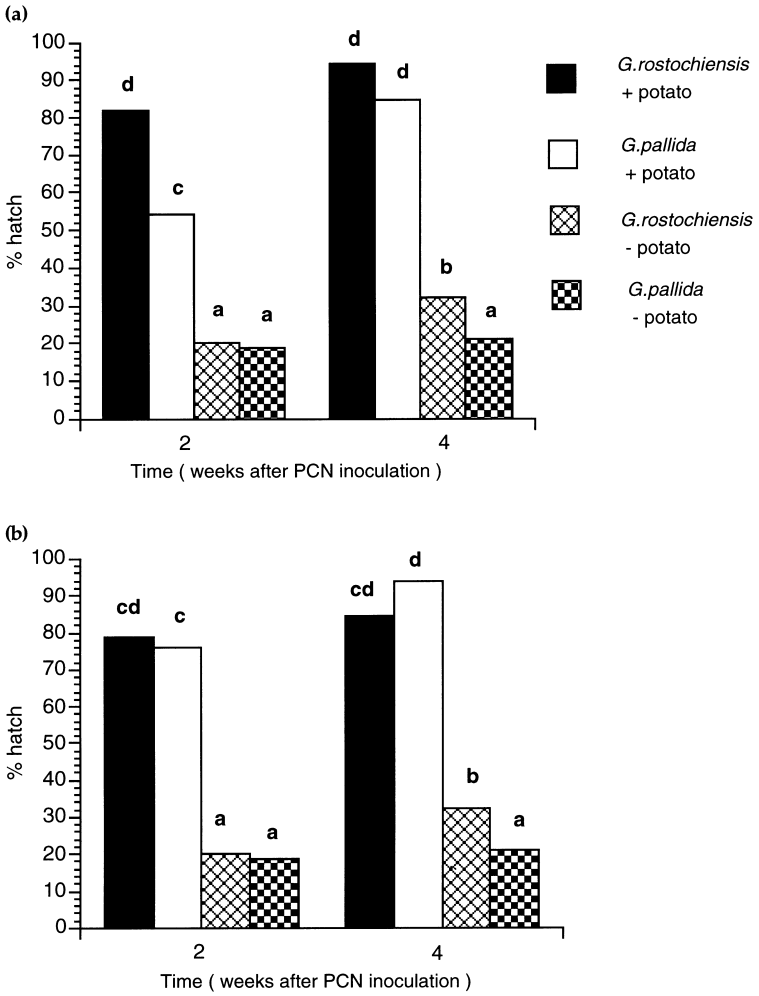

Fig. 1. Spontaneous and potato-induced in-soil hatch of PCN in (a) the absence of and (b) the presence of VAM. Any two samples (within each figure) sharing a common letter were not significantly different (p>0.05) using the Tukey test; n=6 for each sample.

percentage hatch of *G. pallida* in the presence of the leachate from non-mycorrhizal plants collected after one week was significantly less than the hatch in the presence of the mycorrhizal-inoculated leachate (Fig. 2). At week 2, this difference in hatch, though still apparent, was not significant (Fig. 2).

3.4. Effect of VAM on in-soil PCN hatch in the presence of a non-host plant

A second in-soil experiment was then conducted to determine whether the same effects of VAM on *G. pallida* hatch occurred when cysts were incubated with a non-host plant (strawberry). Compared to the spontaneous hatch of *G. pallida* (in the absence of a strawberry plant), the presence of strawberry plants resulted in higher hatch (Fig. 3); the effect was only significant in the presence of Vaminoc-inoculated plants, but no significant difference was observed between the hatch induced by inoculated and uninoculated strawberry plants (Fig. 3).

3.5. Data analysis

In each experiment, the variables approximated to a normal distribution. Analysis was carried out by

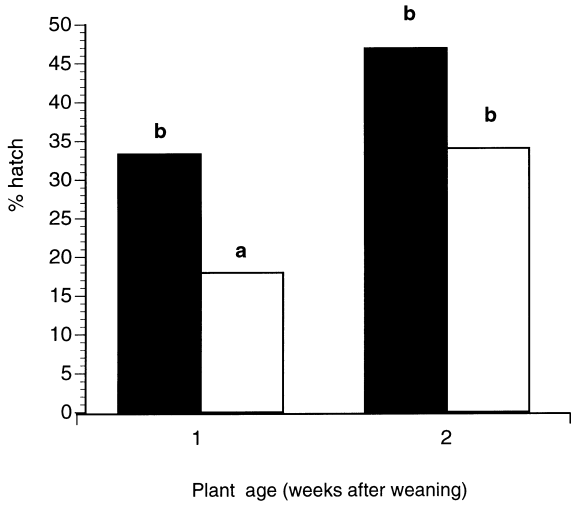

Fig. 2. In vitro hatching activity of *G. pallida* in the presence of PRL from Vaminoc-inoculated (■) and -uninoculated (□) potato plants. Any two samples sharing a common letter were not significantly different ($p>0.05$) using the Tukey test; $n=6$ for each sample.

parametric analysis of variance, with the Tukey test used to carry out multiple comparisons.

4. Discussion

Although the potato plants had been pre-colonised with VAM before PCN was introduced, there was a small decrease in the level of VAM colonisation of the potato root system in the presence of PCN (Table 1). Although the difference was significant only at harvest 3, the observation that the minus-PCN samples exhibited increased VAM colonisation at each of the four independent harvests (Table 1) suggests that PCN infestation somehow interrupted mycorrhizal colonisation.

Mycorrhizal inoculation increased the potato-induced hatch of *G. pallida* by almost 25% in the first two weeks of plant growth, but had no significant effect on *G. rostochiensis* hatch. The fact that the mycorrhizal-stimulation of *G. pallida* hatch was observed both in-soil and in vitro, using independent batches of plants, indicates that this effect is not an artefact. The fact that the same leachate resulted in hatch stimulation in only *G. pallida* means that the effect was not merely a reflection of any change in root growth, but that it was due to altered production of *pallida*-selective hatching chemicals.

The most likely scenario involves the mycorrhizal stimulation of production of either HFs or HSs, either as a result of increased production of potato chemicals or the generation of specific mycorrhizal hatching chemicals. The observation that the effect was not observed in the non-HF-producing species, strawberry, suggests (but does not prove) that the mycorrhizal effect is not due to the production of fungal HFs, but could be due to the increased production of potato HFs or HSs, or the synthesis of novel fungal HSs.

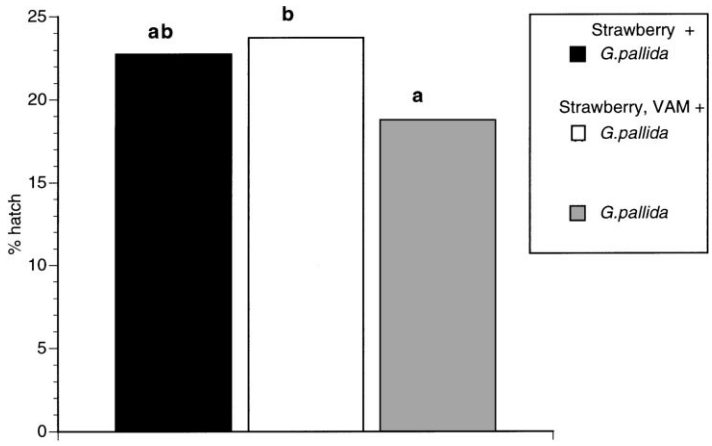

Fig. 3. In-soil hatch of *G. pallida* in the presence of Vaminoc-inoculated and -uninoculated strawberry plants. Any two samples sharing a common letter were not significantly different ($p>0.05$) using the Tukey test; $n=6$ for each sample.

To distinguish between these possibilities, the hatching chemicals present in root leachates from mycorrhizal and non-mycorrhizal potato plants will need to be resolved and compared (Devine et al., 1996; Byrne et al., 1998). 'Vaminoc' is a cocktail of three *Glomus* spp. (I. Arias, MicroBio, pers. comm.). It is possible that other VAM would have different effects on the potato–PCN interaction.

The in-soil hatching behaviour of the two PCN species closely resembled that reported from field observations: *G. pallida* generally exhibits less spontaneous hatch and slower potato-induced hatch than *G. rostochiensis* (Whitehead, 1992). This similarity indicates that the results from the in-soil studies could be extrapolated (albeit with caution) to the field. The delayed hatch of many *G. pallida* populations has been associated with the relatively poor control of this PCN species by granular nematicides such as aldicarb (Whitehead, 1992); by the time *G. pallida* hatches, the short half-life of the nematicide (Whitehead, 1992) means that it is present at sub-optimal concentrations. If the mycorrhizal stimulation of *G. pallida* hatch observed in-soil can be confirmed in the field, the resulting synchronisation of hatch of the two PCN species by mycorrhizal inoculation could result in more effective nematicidal control of *G. pallida*, offering the prospect of integrated biological–chemical control of *G. pallida*.

These studies support the report of Devine et al. (1996) that some of the HFs in potato root leachate may be of microbial origin. Tsutsumi (1976) suggested that the seasonal flushes of spontaneous hatch of PCN in fallow soil were the result of microbial activity in the soil, while Racke and Sikora (1992) and Carroll (1995) have demonstrated that free-living and rhizosphere bacteria, respectively, can affect potato-induced PCN hatch. The current study is the first report of a fungal involvement in the chemical interaction between potato and PCN. Apart from any potential role in biological control of PCN, this study emphasises the complex nature of this interaction in the soil.

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