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Enhancement of the efficacy of a carbamate nematicide against the potato cyst nematode, *Globodera pallida*, through mycorrhization in commercial potato fields

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Abstract: Two experiments were conducted over 2 years in commercial potato fields in Shropshire, UK, to evaluate the compatibility of the nematicide aldicarb with commercial inocula of arbuscular mycorrhizal fungi (AMF) in the control of the potato cyst nematode *Globodera pallida*. The AMF used were Vaminoc (mixed-AMF inoculum), *Glomus intraradices* (BioRize BB-E) and *G. mosseae* (isolate BEG 12). In the absence of AMF, the in-soil hatch of *G. pallida* increased 30% ($P < 0.01$) from wk-2 to wk-4 after planting. Inoculation of physiologically-aged potato (cv. Golden Wonder) tubers with AMF eliminated this delay in *G. pallida* hatch by stimulating a mean increase of 32% ($P < 0.01$) in hatch within 2 wk after planting. In the aldicarb-treated plots in Experiment 1, *G. pallida* multiplication rate was 38% lower ($P < 0.05$) in roots of AMF-inoculated than noninoculated plants, but in Experiment 2, this effect was slightly lower ($P = 0.07$). In these plots, the single AMF inocula showed also a weak trend ($P = 0.10$) towards greater tuber yields relative to their noninoculated counterparts. Mycorrhization therefore appears to enhance the efficacy of carbamate nematicides against *G. pallida* and consequently more research is proposed to validate these findings and fully explore the potential of this model.

Key words: integrated pest management, potato cyst nematode, *Globodera pallida*, arbuscular mycorrhizal fungi, *Glomus* spp., interaction, aldicarb, hatch, multiplication, *Solanum tuberosum*.

The white and golden potato cyst nematodes (PCN), *Globodera pallida* (Stone) Behrens and *G. rostochiensis* (Wollenweber) Behrens, respectively, are damaging and economically important pests of the potato (*Solanum tuberosum* L.) crop in many potato-growing regions of the world. In Europe, yield losses to PCN have been estimated at *ca* €300m p.a. (Deliopoulos et al., 2007) and in the UK alone in excess of £50m p.a. (Bhattarai et al., 2009).

The two species have been recorded in most (*G. pallida*) or all (*G. rostochiensis*) EU Member States which have carried out official tests to species level, but there is considerable variation in PCN incidence by country (Pickup, 2007). In the USA, PCN was identified in the 1940s on Long Island, New York, and soon after became the subject of quarantine regulations under the Golden Nematode Act (Brodie and Mai, 1989). Since 2003, *G. rostochiensis* is among the 22 federally regulated organisms in the USA (Code of Federal Regulations [CFR], 2003). As regards *G. pallida*, which is the species of interest in this study, its presence was confirmed for the first time in the USA in April 2006 in a soil sample from a potato processing facility in Idaho. This was the only positive sample identified in the Cooperative Agriculture Pest Survey (2000-2006) carried out nationwide by the Animal and Plant Health Inspection Service of the United States Department of Agriculture (USDA). However, within 6 months since its first detection, nine fields of close proximity to each other were found positive to *G. pallida* in Bingham County, Idaho (USDA, 2009). In Canada, *G. pallida* has been recorded in a relatively small geographical area of Newfoundland (Brodie, 1998).

A widely used and heavily relied-upon component of PCN management is the use of granular, non-fumigant, nematicides (Evans and Haydock, 2000), which are applied at planting (Haydock and Evans, 1998). Such nematicides used in the UK for PCN control have included aldicarb and oxamyl (carbamates) and fosthiazate and ethoprophos (organophosphates) (Haydock et al., 2006). The nematicide aldicarb (Temik 10G, Bayer CropScience, Cambridge, UK), which was used in the present study, is currently registered for PCN control on potatoes in seven States in the USA (Oregon, Idaho, Washington, Montana, northern Florida, and parts of Utah and Nevada) (Environmental Protection Agency [EPA], 2007), while in the UK its "essential uses" status ended at the end of 2007 (European Community [EC], 2003).

In the UK, farmers traditionally follow an integrated approach to control PCN, which involves the use of nematicides, crop rotation and resistant cultivars (Haydock and Evans, 1998). All these methods have low effectiveness against *G. pallida*, which has become the predominant species in England and Wales with presence in over 90% of the PCN-infested fields (Minnis et al., 2002), while its incidence in Scotland is increasing (Trudgill et al., 2003). Turner et al. (2006) stressed that the increasing incidence of *G. pallida* in the UK in recent years has been such that many fields are now considered unproductive for potato cropping. The prospects for *G. pallida* management do not look promising and the current epidemic may get more serious in the future (Trudgill et al., 2003) if the limitations set by the potato industry on nematicide usage (Haydock and Evans, 1998), combined with the reduction in nematicide availability, continue. As has been described by Deliopoulos et al. (2007), a major constraint in the effective use of granular nematicides,

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such as aldicarb, against *G. pallida* arises from the short persistence of these products in the soil and the late hatching pattern of this nematode species. The consequence is that the majority of *G. pallida* second-stage juveniles (J2) hatch after most of the active substance has been degraded to sub-optimal concentrations (Whitehead, 1992).

It is therefore imperative to develop alternative and more effective integrated pest management (IPM) methods of controlling *G. pallida* in which reduced nematicide inputs must be a prerequisite. In this respect, a range of novel *G. pallida* control techniques have been investigated in recent years and more are being sought as research in the field is ongoing; examples include trap cropping with *Solanum sisymbriifolium* (Timmermans et al., 2006), the use of the resistant species *Solanum canasense* (Castelli et al., 2006) and biological control using *Pochonia chlamydosporia* (Tobin et al., 2008).

Hatch is a critical survival stage in the PCN life cycle and Nordmeyer (1990) identified it as a possible target for the development of novel control agents. This is due to the near-absolute reliance of PCN on hatching factors (HFs, host-specific semiochemicals present in potato root leachate [PRL]; Devine et al., 1996) to trigger hatch and to the limited viability of J2 in soil after they have hatched in the absence of the host plant (Storey, 1984; Robinson et al., 1987; Perry, 1997). Consequently, any modification of the hatching activity of PRL towards PCN would be expected to have a large effect on PCN hatching behavior. The PCN hatching mechanism has been extensively studied (Perry, 1989, 1997; Jones et al., 1998) opening up new prospects for PCN management using novel, hatch-based control methods.

Evidence exists that rhizobacteria and arbuscular mycorrhizal fungi (AMF) play an important role in affecting in-soil PCN hatch by altering the production of HFs in PRL (Jones et al., 1998; Ryan and Jones, 2003, 2004; Deliopoulos et al., 2007). The reason why AMF have attracted interest as possible PCN hatching stimulators and/or potential PCN biocontrol agents (both associated with increased nematicide effectiveness), is because they occupy regions of the root system similar to those colonized by PCN (Ingham, 1988) and hence there is potential that they may interfere with the PCN life cycle.

In light of this speculation, a range of *in vitro* and in-soil studies were undertaken to investigate the various aspects of the PCN-AMF interaction under potato plants (microplants and seed-tuber-derived) in the presence or absence of the nematicide aldicarb. The results have been consistent in that PRL from mycorrhizal treatments produced a *G. pallida*-specific increase in total percentage hatch and stimulated the production of an increased amount of *G. pallida*-active HFs compared with PRL from control plants (Ryan et al., 2000; Ryan and Jones, 2003; Ryan et al., 2003; Ryan and Jones, 2004; Deliopoulos, 2004; Deliopoulos et al., 2007, 2008).

The results of the experiments described above provided strong evidence that the effectiveness of granular nematicides may be improved by incorporating an application of commercial AMF inocula at the time of planting. However, before AMF could be recommended as part of a nematicide-based IPM strategy to control *G. pallida*, it was necessary to assess their efficacy in commercial potato fields. The use of commercial field settings instead of research stations was a strong part of this research. Such stations normally do not have the typical cropping history found in commercial fields, therefore limiting the practical utility of the results. The sites chosen here were indicative of commercial potato production so as to provide important practical information that can also be utilized in other commercial situations.

Any role of AMF inoculation of potato on *G. pallida* control must be robust and effective under a variety of conditions and consequently the experiments were repeated at two different sites. The principal aim of the presented research was to investigate whether field application of AMF inocula at planting would improve the efficacy of the non-persistent nematicide aldicarb to control *G. pallida*.

Materials and Methods

Plant material: Certified seed potatoes (CC grade, 35-45 mm) of the late maincrop cultivar Golden Wonder were used in both experiments. The seed potatoes were physiologically-aged for 4 wk at 15°C in the light prior to planting until they produced strong, sturdy sprouts approximately 2.5 cm long.

Estimation of Globodera pallida *population density and percentage hatch:* Bulked samples of *ca* 2.5 kg consisted of 60 soil cores (2.5-cm-diam. and 20-cm deep) were taken from the two harvest rows of each replicate plot separately using a cheese-style auger with a half-cylindrical blade, immediately after planting and one day after harvest to determine the initial (Pi) and final (Pf) *G. pallida* population densities and the *G. pallida* multiplication rate (Pf/Pi). The soil samples were collected in linen bags and dried at 25°C for 5 days in a ventilated cupboard equipped with tubular heaters and a fan. Each air-dried sample was then passed through a 4 mm sieve to remove stones and coarse gravel, thoroughly mixed, and a 200 g sub-sample was taken from each sample. A Fenwick can (Fenwick, 1940) was used to extract the cysts from the 200 g soil sub-sample. The number of cysts and cyst contents were estimated using standard methods (Southey, 1970). Nematode population densities were expressed as eggs/g soil.

Similar-sized soil samples were also taken from each plot at 2 and 4 wk after planting in Experiment 1 and at 6 and 8 wk after planting in Experiment 2 to determine the percentage of hatched J2. The soil samples were then air-dried and cysts were extracted as described above. The extracted cysts were then transferred in watch glasses and incubated in distilled water at 22°C for one week. They were then placed in 0.05% (w/v)

aqueous Meldola Blue solution (Sigma-Aldrich, Poole, UK) for one further week and finally immersed in distilled water for 24 hr to remove excess stain. The number of cysts, viable, non-viable and empty eggs (each representing a hatched J2; Ryan et al., 2003) were determined as described by Shepherd (1986). The percentage hatch of the viable eggs was determined using the formula:

$$\% \text{ viable egg hatch} = ([\text{number of hatched eggs}] \div [\text{number of hatched eggs} + \text{viable full eggs}]) \times 100$$ (Byrne et al., 1998).

Since the cysts used in the experiments were field populations, any empty eggs and viable full eggs counted here would have originated from cysts of different ages and not only from that season's generation. Because, however, the whole experimental site had similar cropping, populations were expected to be variable but uniform across treatments. Potatoes had been previously planted in these fields and hence *G. pallida* cysts would expect to contain viable unhatched eggs because of slow utilization of lipid reserves in the absence of host roots (Turner, 1985).

A suspension of 50 crushed PCN cysts was used as a source of template DNA for the polymerase chain reaction (PCR) to identify the PCN species present (Ibrahim et al., 2000).

Arbuscular mycorrhizal fungi: In both experiments, the AMF used were Vaminoc, a commercial mixed-isolate of three *Glomus* species, and two of the Vaminoc component isolates, *Glomus intraradices* (BioRize BB-E) and *Glomus mosseae* (isolate BEG 12). The AMF inocula had a concentration of 30 infective propagules per gram inoculum and were formulated (as described in Deliopoulos et al., 2007) and supplied by Dr. B. Blal (BioRize, Dijon, France). Each tuber was inoculated with Vaminoc or *G. intraradices* or *G. mosseae* granular inoculum at a rate of 10 g/tuber in Experiment 1 and 5 g/tuber in Experiment 2. For this purpose the required dose of AMF inoculum was placed into the planting holes to a depth of 15 cm just before planting the tuber. For control plants, no AMF inoculum was placed in the planting hole. The higher inoculum dose used in Experiment 1 compared with Experiment 2 was justified by published evidence suggesting that in high-P soils (such as Experiment 1 here), AMF can still be efficient if high levels of mycorrhizal inoculum are used, providing that such soils have good P fixing ability (Plenchette and Fardeau, 1988; Plenchette et al., 2005).

Trial sites and design: The field sites were situated near Harper Adams University College, Shropshire, UK, on sandy loam soils. A 4-year crop rotation was adopted at both sites comprising cereal-cereal-sugar beet-potato. In Experiment 1 (conducted in 2001; high-nutrient/low-infestation trial) the equivalent of 175 kg/ha N and 150 kg/ha K_2O was applied by hand prior to planting onto the formed seedbed. Soil analysis at planting indicated that the P, K and Mg concentrations were 101 ppm, 226 ppm and 65 ppm, respectively, and that the soil pH was 5.8. In Experiment 2 (conducted in 2002; low-nutrient/high-infestation trial), the concentrations of P, K and Mg in the soil at the time of planting were 40 ppm, 220 ppm and 61 ppm, respectively, pH 6.5, and no fertilizer was added at planting. Soil analysis was conducted by Directlaboratories (Wolverhampton, UK).

The experimental design in both trials was a 2 x 4 factorial split plot. The factor nematicide (two levels: presence, absence) was allocated to the two main plots and the treated plot received the equivalent of 33.6 kg/ha of the nematicide aldicarb as Temik 10G (10% a.i. w/w granules: Bayer CropScience, Cambridge, UK). The aldicarb granules were broadcast-applied and then incorporated on the day the crops were planted according to the manufacturer's recommendations using a tractor mounted rotavator to a depth of approximately 20 cm. The factor AMF (four levels: control, Vaminoc, *G. intraradices* and *G. mosseae*) was allocated as five randomized blocks to the 20 split plots in each main plot. Thus, there were eight treatments in total, with five replicate plots per treatment, giving a total of 40 plots for the entire experiment. Each split plot consisted of two rows 5 m long and 1.83 m (one bed) wide. The sprouted tubers (16 per row) were planted by hand to a depth of 15 cm with 30 cm in-row spacing. The two main plots were divided by an unplanted guard bed 1.83-m wide for the purpose of minimizing the edge effect (Langton, 1990), while the distance between subsequent plots was 50 cm.

Quantification of root colonization by AMF: At 4 wk after planting in both experiments, two whole plants were removed from the end 1 m of each plot (one plant from each row) and their root systems were washed, excised and weighed. Following this, roots from each plant were cut into approximately 2 cm pieces and a 2 g fresh weight sub-sample was taken per plant and preserved in 40% (v/v) formalin acetic alcohol (FAA) in 30 ml glass specimen tubes until needed. For assessing mycorrhizal colonization, each root sub-sample was stained with 0.05% (w/v) Trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and distilled water; Brundrett et al., 1994) and the percentage root colonization by AMF was determined using the grid line intersect method of Giovannetti and Mosse (1980).

Root invasion by Globodera pallida*:* In Experiment 2, another 2 g fresh weight sub-sample was taken from the roots of each of the same two plants per plot as in AMF root colonization and preserved in FAA until assessment of *G. pallida* root invasion was made. Each 2 g root sub-sample was then stained in 0.05% w/v acid fuchsin (Bridge et al., 1982) and the number of developing juvenile nematodes (all stages) was determined as described by Hooper (1986). The result was expressed as number of feeding nematodes/g of root at 4 wk after planting.

Phosphorus analysis of plant material: At 6 wk after planting in Experiment 2, one whole plant was removed from

the first 1 m of each of the two rows of each plot giving two plants per plot. The plants were transferred to the laboratory in plastic bags moistened with distilled water to prevent wilting. In the laboratory the plants were thoroughly washed with 0.1% (v/v) 'Teepol' detergent for 30 sec and then rinsed three times in deionized water for 20 sec each time. Each plant was then dissected into component parts. The first fully expanded composite leaf (fourth from the top of the plant) and the main stem were selected from each plant for P analysis. The selected plant material (leaf and stem separately) was then dried to constant weight in a fan ventilated oven at 75°C for 36 hr, weighed and milled to 1 mm. After milling, the leaf and stem dry matter were bulked for each plant and 1 g dry matter sub-sample was taken for P analysis. In total, 80 samples were analyzed (2 plants per plot × 5 replicate plots × 8 treatments). The concentration of P in the plant material was measured by spectrophotometric determination of the phospho-vanado-molybdate complex (Ministry of Agriculture, Fisheries and Food [MAFF], 1986). Two readings were made per sample and the result was expressed as mg P/g plant dry matter.

Harvest: Two weeks before harvest in Experiments 1 and 2, the haulms were desiccated with Reglone® at 0.8 liters a.i./ha (200 g/liter diquat, Syngenta Ltd, UK). All plots were harvested using a tractor-mounted, two-row potato spinner followed by forking, and yield was assessed from the middle 4 m of each of the two rows per plot, giving a harvest area of 7.32 m^2. Harvest was carried out 147 days after planting (DAP) in Experiment 1 and 99 DAP in Experiment 2. The tubers were graded into three size classes: < 45 mm, 45-65 mm and > 65 mm and then counted and weighed separately in each class. Experiment 2 was harvested early (48 days earlier than Experiment 1) so that the tubers had not fully matured and consequent yield was depressed.

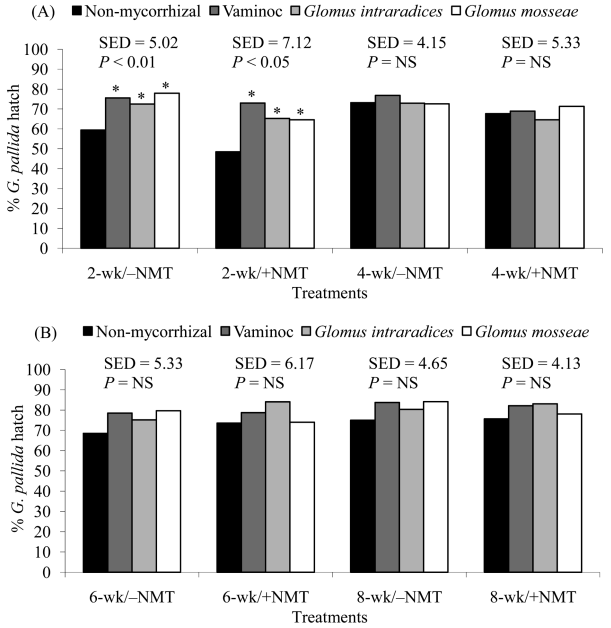
Data analysis: Data were analyzed by parametric interaction analysis of variance (ANOVA) or unpaired *t*-test using the software Genstat Release 10.1, Lawes Agricultural Trust (Rothamsted Experimental Station, UK). Where necessary, data were transformed to obtain homogeneity of variance and the most appropriate transformation in each case was applied to the data prior to analysis. Simple one-way ANOVA was also performed in the data of each main plot (i.e., plus/minus nematicide) in order to analyze further the effect of AMF as a single factor on the parameters assessed. When the ANOVA probability was found to be significant, Fisher's protected Least Significant Difference (LSD) was used to conduct multiple comparison tests between the treatment means.

Results

PCN species identification: The results of the PCR tests determined that the field sites in both experiments contained a population of pure *G. pallida*.

In soil hatch of Globodera pallida*:* In Experiment 1, AMF inoculation stimulated significant increases in percentage *G. pallida* hatch, relative to noninoculated plants, only at 2 wk after planting in both the presence ($P < 0.01$) and absence ($P < 0.05$) of aldicarb without significant differences between the three AMF treatments (Fig. 1A). The mean increase in percentage hatch in AMF-inoculated plots compared with controls was 32% at 2 wk after planting.

The significant time × AMF interaction ($P < 0.01$) combined with the lack of a three-way (time × nematicide × AMF) interaction on percentage hatch (Experiment 1) supported the finding that AMF inoculation stimulated increased *G. pallida* hatch relative to noninoculated plants only at 2 wk after planting, both in the absence ($P < 0.01$) and presence ($P < 0.05$) of aldicarb. Four weeks after planting, the hatching response of *G. pallida* from AMF-inoculated and noninoculated plants was statistically similar and from 2 to 4 wk only under non-mycorrhizal plants was the hatch



ANOVA summary for percentage hatch of *Globodera pallida* (error df = 64)

	Experiment 1 (Fig. 1A)		Experiment 2 (Fig. 1B)	
Source of variation	*F*-ratio	SED	*F*-ratio	SED
Time (TIM) $^{df=1}$	4.50 *	1.847	4.26 *	1.814
Nematicide (NMT) $^{df=1}$	14.76 ***	1.847	0.09 ^{NS}	1.814
Mycorrhiza (AMF) $^{df=3}$	7.25 ***	2.612	3.88 *	2.565
TIM × NMT $^{df=1}$	0.56 ^{NS}	2.612	0.78 ^{NS}	2.565
TIM × AMF $^{df=3}$	5.17 **	3.694	0.09 ^{NS}	3.628
NMT × AMF $^{df=3}$	0.13 ^{NS}	3.694	1.94 ^{NS}	3.628
TIM × NMT × AMF $^{df=3}$	1.07 ^{NS}	5.224	0.12 ^{NS}	5.131

Asterisks above columns denote significant increase relative to the non-mycorrhizal using the Least Significant Difference test at the 5% level. SED = Standard error of difference between means. df = Degrees of freedom. NS = not significant. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

Fig. 1. Percentage in-soil hatch of *Globodera pallida* eggs in the absence (–NMT) or presence (+NMT) of the nematicide aldicarb, under non-mycorrhizal potato plants and under potato plants inoculated with the arbuscular mycorrhizal fungi (AMF) isolates Vaminoc, *Glomus intraradices* or *Glomus mosseae*. (A) Experiment 1: 2 and 4 wk after planting, (B) Experiment 2: 6 and 8 wk after planting.

of *G. pallida* significantly increased (wk-2: 54%, wk-4: 70.4%; $P < 0.001$). Nematicide application had a significant ($P < 0.001$) main effect on percentage hatch, reducing it from 72.6% in nontreated plots to 65.5%.

In Experiment 2, there were no significant differences in percentage *G. pallida* hatch between AMF-inoculated and noninoculated plots at either observation date (i.e., 6 and 8 wk after planting) in either the presence or absence of nematicide (one-way ANOVA tests; Fig. 1B).

The 3-way ANOVA for Experiment 2 at the bottom of Fig. 1 illustrates that while incorporation of the nematicide aldicarb did not significantly affect *G. pallida* hatch, both AMF and time produced significant main effects on percentage hatch. In particular, hatch under AMF-inoculated plants (mean of 80%) was significantly higher ($P = 0.01$) than under noninoculated plants (73.2%) and was greater at 8 wk after planting compared with 6 wk after planting (means of 80.3% and 76.5%, respectively; $P < 0.05$).

The overall mean of percentage hatch of *G. pallida* in Experiment 2 (averaged over all factors, i.e., time, AMF and nematicide) was 78.4% and it was significantly greater than the respective mean (69.1%) in Experiment 1 ($t = 6.23$, df = 158, $P < 0.001$).

Globodera pallida *population density:* The mean initial infestation levels ± SE were 17 ± 1.7 eggs/g soil in Experiment 1 and 89 ± 6.1 eggs/g soil in Experiment 2.

When averaged over all AMF treatments, nematicide application in Experiment 1 had a highly significant main effect on the Pf/Pi ratio, which was almost halved compared with the aldicarb-nontreated plots ($P < 0.001$; Table 1). However, aldicarb failed to control the *G. pallida* population increase in the noninoculated plots, as the means of Pf/Pi were almost identical for aldicarb-treated and aldicarb-nontreated plots (8.7 and 8.5, respectively; Table 1). There was a significant interaction between AMF and nematicide on *G. pallida* reproduction ($P < 0.05$; Table 1). In particular, in comparison with noninoculated plots, Pf/Pi in AMF-inoculated plots tended to be higher (45%) in the absence of aldicarb but was significantly lower (38%) when nematicide was added to the plots ($P < 0.05$; Table 1).

In Experiment 2, aldicarb treatment had a significant controlling effect on Pf/Pi ($P < 0.001$; Table 1), reducing it from 2.38 averaged over all aldicarb-nontreated plots to 0.96 averaged over all treated plots. The simple ANOVA for Pf/Pi in the aldicarb-treated block revealed differences between treatments that approached significance ($P = 0.066$; Table 1) and a high coefficient of variation (CV = 23.9%). In particular, nematode reproduction was decreased by almost 30% on mycorrhized plants of all three isolates as compared with noninoculated aldicarb-treated plants (Table 1).

Root invasion by Globodera pallida*:* Microscopic examination of the root tissues 4 wk after planting in Experiment 2 revealed that AMF interacted significantly with nematicide ($P < 0.001$) to affect the numbers of juveniles invading the root system (Table 2). Although both factors also produced a significant main effect, this effect was much stronger for aldicarb than for AMF as demonstrated by the *F*-ratios of 186.8 and 3.2, respectively. Overall, when compared with nontreated plots, aldicarb application suppressed the number of juvenile nematodes invading the potato roots to almost one-third (from 972 to 338 nematodes/g root). Nevertheless, analysis of the nematicide × AMF interaction indicated that the differences in the numbers of invading juveniles in potato roots between aldicarb-nontreated and aldicarb-treated plots were larger in AMF-inoculated plants than in noninoculated

TABLE 1. The effect of applying both arbuscular mycorrhizal fungi (AMF) and the nematicide (NMT) aldicarb at potato planting on *Globodera pallida* multiplication rate (Pf/Pi) (based on eggs/g soil). Means of values transformed by $x^1 = \log_{10}(x + 1)$ are presented (back-transformed means in parentheses).

Globodera pallida multiplication rate (Pf/Pi) [†]				
	Experiment 1		Experiment 2	
AMF	without aldicarb	with aldicarb	without aldicarb	with aldicarb
Non-AMF	0.99a (8.73)	0.98b (8.49)	0.51a (2.25)	0.37b (1.34)
Vaminoc	1.09a (11.24)	0.87ab (6.49)	0.53a (2.42)	0.26a (0.83)
G. intraradices	1.18a (14.09)	0.69a (3.95)	0.55a (2.53)	0.27a (0.84)
G. mosseae	1.13a (12.63)	0.81a (5.39)	0.52a (2.32)	0.26a (0.84)
SED $^{df = 16}$	0.141 ^{NS}	0.085 *	0.066 ^{NS}	0.044 ^{v}

ANOVA summary for multiplication rate (error df = 32)				
	Experiment 1		Experiment 2	
Source of variation	*F*-ratio	SED	*F*-ratio	SED
Nematicide (NMT) $^{df = 1}$	19.80 ***	0.144	72.05 ***	0.067
Mycorrhiza (AMF) $^{df = 3}$	0.13 ^{NS}	0.209	0.60 ^{NS}	0.096
NMT × AMF $^{df = 3}$	2.92 *	0.308	1.36 ^{NS}	0.138

[†] Means sharing a common letter in the same column were not significantly different at the 5% (Experiment 1: without/with aldicarb, Experiment 2: without aldicarb) or 7% (Experiment 2: with aldicarb) levels according to the Least Significant Difference test. SED = Standard error of difference between means. df = Degrees of freedom. NS = not significant. * $P < 0.05$. ^{v} $P = 0.066$. *** $P < 0.001$.

TABLE 2. Total number of feeding *Globodera pallida* nematodes/g of root at 4 wk after planting (Experiment 2) in non-mycorrhizal potato plants and in potato plants inoculated with the arbuscular mycorrhizal fungi (AMF) isolates Vaminoc, *Glomus intraradices* or *Glomus mosseae*, in the absence or presence of the nematicide aldicarb (NMT). Means of values transformed by $\log_{10}$ (x) are presented (back-transformed means in parentheses).

	Root invasion by *Globodera pallida* (nematodes/g of root) [†]	
AMF inoculum	Without aldicarb	With aldicarb
Non-AMF	2.91a (806)	2.72c (522)
Vaminoc	3.03b (1078)	2.54b (348)
G. intraradices	2.99ab (985)	2.52b (333)
G. mosseae	3.02b (1043)	2.33a (214)
SED $^{(df = 16)}$	0.046 [y]	0.083 **

	ANOVA summary for root invasion (error df = 32)		
Source of variation	*F*-ratio	*Probability*	SED
Nematicide (NMT) $^{df = 1}$	186.79	< 0.001	0.034
Mycorrhiza (AMF) $^{df = 3}$	3.16	< 0.05	0.048
NMT × AMF $^{df = 3}$	9.32	< 0.001	0.067

[†] Means sharing a common letter in the same column were not significantly different at the 6% (without aldicarb) or 5% (with aldicarb) levels according to the Least Significant Difference test. SED = Standard error of difference between means. df = Degrees of freedom. [y] $P = 0.060$. ** $P < 0.01$.

plants. In particular, noninoculated plant roots taken from plots treated with aldicarb contained 65% of the total number of feeding nematodes found in roots from plots not treated with aldicarb. On the other hand, roots of plants of AMF-inoculated, aldicarb-treated plots, contained only 32% (Vaminoc), 34% (*G. intraradices*) and 21% (*G. mosseae*) of the number of nematodes/g of root measured in their aldicarb-nontreated counterparts (Table 2).

In comparison of the individual treatments, there was a trend for increasing root invasion by *G. pallida* following AMF inoculation in the absence of aldicarb ($P = 0.06$; Table 2). The largest increase was exhibited by the mixed-isolate Vaminoc inoculum, with 272 (34%) more feeding nematodes/g root compared with the corresponding number found on roots of noninoculated plants. When plots were treated with aldicarb, inoculation with all three AMF resulted in significant reductions in *G. pallida* root invasion ($P < 0.01$), which ranged from 33% (Vaminoc) to 59% (*G. mosseae*) (Table 2).

Root length colonization by AMF: There was no mycorrhizal colonization detected in the noninoculated plants indicating the absence of native AMF in the soil. In neither of the two trials, with or without aldicarb, was there a significant difference between the three AMF treatments on percentage root length colonization. Application of the nematicide aldicarb did not affect mycorrhization in either of the experiments and also neither the AMF main effect nor the nematicide × AMF interaction were significant. The overall means of percentage root length colonization by the AMF isolates Vaminoc, *G. intraradices* and *G. mosseae*, averaged over the two levels of the nematicide factor, were 37.3%, 34.2% and 34.7% (CV = 26.1%), respectively, in Experiment 1 and 49.8%, 46.6% and 46.2% (CV = 31.3%) in Experiment 2. The respective grand means of 35.4% and 47.5% were significantly different ($t = 4.01$, df = 58, $P < 0.001$).

Phosphorus analysis of plant material: The concentrations of P in the plant tissues were within the range of 1.14 to 1.54 mg/g dry matter (CV = 13.4%). Arbuscular mycorrhizal fungi, as a main effect, did not influence P tissue content significantly, but conversely, nematicide treatment had a highly significantly effect ($P < 0.001$), increasing the P content by approximately 18%. The nematicide × AMF interaction on P content was not significant.

Tuber yield: The crop in Experiment 2 was harvested 48 days earlier than in Experiment 1. By this time, tubers had not fully matured and consequently yield was depressed. The nematicide effect on total tuber yield was very significant in both trials (Experiment 1: $P < 0.01$; Experiment 2: $P < 0.001$) and independent of the AMF treatment (i.e., no nematicide × AMF interaction) (Table 3). The overall increase in mean total yield as a result of aldicarb application, averaged across the factor AMF, was 6.3 tonnes/ha (or 28%) at 147 DAP in Experiment 1 and 5.3 tonnes/ha (or 39%) at 99 DAP in Experiment 2. The average proportion of tubers of ware grade was two-thirds of the total tuber yield in Experiment 1 and one-fourth in Experiment 2, reflecting the shorter period of growth in the latter. The grand means for total yield in Experiments 1 and 2 were 25.4 tonnes/ha and 16.1 tonnes/ha ($t = 8.36$, df = 78, $P < 0.001$), respectively.

Although mycorrhization as a main effect did not influence yield significantly, aldicarb-treated plots that received at planting 10 g/tuber of the mycorrhizal inocula *G. intraradices* or *G. mosseae* showed a trend ($P = 0.097$) for higher yields (both over 30 tonnes/ha) than those plots that did not receive AMF treatment at planting (27.1 tonnes/ha) (Table 3). In plots without aldicarb in Experiment 1, the mean values for total yield were between 2-4 tonnes/ha greater in AMF-inoculated plots than in noninoculated controls, but this was not significant. In fact, in Experiment 1, despite opposite trends in *P*-values (0.866 and 0.097, respectively), percentage yield increase in the AMF-inoculated plots (relative to the noninoculated) was greater in the aldicarb-nontreated (13.1%) than in the aldicarb-treated plots (7.3%) (Table 3). However, the CV in the former was more than three times higher (17.7%) as that in the latter (5.1%). This variation in CV values of total tuber yield between the two data sets suggests that in the absence of aldicarb, any differences in means would have had to be much larger than in its presence to attain statistical significance. Total tuber yields in Experiment 2 in plots that had received an AMF inoculum at planting were statistically similar to

TABLE 3. Total tuber yield (tonnes/ha) of non-mycorrhizal potato plants (cv. Golden Wonder) and of potato plants inoculated with the arbuscular mycorrhizal fungi (AMF) isolates Vaminoc, *Glomus intraradices* or *Glomus mosseae*, in the absence or presence of the nematicide aldicarb (NMT). Means of values transformed by square root ($\sqrt{x}$) are presented (back-transformed means in parentheses).

	Total tuber yield (tonnes/ha)			
	Experiment 1		Experiment 2	
AMF	without aldicarb	with aldicarb	without aldicarb	with aldicarb
Non-AMF	4.49 (20.12)	5.20 (27.06)	3.73a (13.91)	4.32a (18.67)
Vaminoc	4.91 (24.07)	5.17 (26.70)	3.60a (12.98)	4.29a (18.41)
G. intraradices	4.70 (22.09)	5.54 † (30.69)	3.72a (13.85)	4.40a (19.32)
G. mosseae	4.83 (23.33)	5.49 (30.17)	3.69a (13.60)	4.38a (19.17)
SED $^{df = 16}$	0.529 ^{NS}	0.173 ^{z}	0.118 ^{NS}	0.165 ^{NS}

	ANOVA summary for total tuber yield (error df = 32)			
	Experiment 1		Experiment 2	
Source of variation	*F*-ratio	SED	*F*-ratio	SED
Nematicide (NMT) $^{df = 1}$	9.92 **	0.197	85.11 ***	0.072
Mycorrhiza (AMF) $^{df = 3}$	0.51 ^{NS}	0.278	0.45 ^{NS}	0.101
NMT × AMF $^{df = 3}$	0.40 ^{NS}	0.394	0.11 ^{NS}	0.143

SED = Standard error of difference between means. df = Degrees of freedom. NS = not significant. † Greater than non-AMF at the 10% level according to the Least Significant Difference test. ^{z} $P = 0.097$. ** $P < 0.01$. *** $P < 0.001$.

yields from noninoculated plots either in the absence or in the presence of the nematicide aldicarb (Table 3).

DISCUSSION

The 30% observed increase in *G. pallida* hatch from wk-2 to wk-4 under noninoculated plants supported earlier findings that there is a significant delay in the early in-soil hatch of this species (Whitehead et al, 1987; Whitehead, 1992). Inoculation of potato with AMF eliminated this early delay in *G. pallida* hatch by stimulating the emergence of 32% extra J2 from cysts at 2 wk after planting, relative to those hatched under noninoculated plants. The level of AMF-induced stimulation of *G. pallida* field hatch within 2 wk after planting was higher than that recorded in sand culture glasshouse studies by Ryan et al. (2003) (14%, Vaminoc only) and Deliopoulos et al. (2008) (10%, mean of Vaminoc, *G. intraradices* and *G. mosseae*), although in both reports the effect was also significant. The consistency of the *G. pallida* hatch-stimulatory effect of AMF in different studies is evidence for the veracity of these observations. However, confirmation of the results obtained here in future field studies with all hatch assessments, namely, at all dates, undertaken in the same experiment would be needed to increase the confidence in that *G. pallida* hatch is affected in field settings.

In Experiment 1, application of aldicarb at the recommended field rate had an inhibitory effect on the early (2-4 wk after planting) hatch of *G. pallida* reducing it by nearly 10% as compared with aldicarb-nontreated plots. On the other hand, in Experiment 2, late (6-8 wk after planting) *G. pallida* hatching was unaffected. Published reports have demonstrated that the time required for aldicarb concentration in the soil to be halved is between 1-3 wk from application (Bromilow et al., 1980; Whitehead, 1992). This short half-life of aldicarb suggests its inhibitory effect on early as opposed to late *G. pallida* hatch might have been related to a possible higher concentration in the soil moisture in the 2-4 wk than the 6-8 wk time point. However, no data on aldicarb decay were collected to support this hypothesis as this was outside the scope of the present studies.

To our knowledge, this is the first report of *G. pallida* hatch inhibition by aldicarb in the field and therefore the exact mechanism has not been established. The inhibitory effect of aldicarb on PCN (referred to as *Heterodera rostochiensis*) hatch has been confirmed by Hague and Pain (1970) and Osborne (1973) in laboratory bioassays. In the latter study, concentrations of aldicarb above 1 ppm inhibited hatch, but when the cysts were transferred to aldicarb-free PRL, hatching was restored. In the same study, it was also observed that hatch inhibition was greater with increasing concentrations (up to 20 ppm) of aldicarb. Hough and Thomason (1975) reported that sublethal aldicarb concentrations (between 0.48-4.8 μg/ml) completely inhibited the in soil hatch of the sugar beet nematode (*Heterodera schachtii*) J2 from encysted eggs. Byrne (1997) observed that the highest-tested aldicarb concentration (5×10^{-2} %) resulted in long-lasting hatch inhibition of both PCN species; on the contrary, low concentrations of aldicarb (5×10^{-6} % and 5×10^{-8} %) were found to stimulate greater levels of PCN hatch than did water controls (Byrne, 1997). In his review of nematicides, Chitwood (2003) reported that the inhibition of acetylcholinesterase by aldicarb impairs the nematode's nervous system and this affects many essential processes of the life cycle including hatching. Haydock et al. (2006) reported that the biochemical effect of aldicarb is reversible at field rate and that at high concentrations nematode

hatch can be disrupted. At high concentrations of aldicarb in the field, it may therefore be possible that the susceptibility of unhatched J2 to paralysis, resulting from the nervous dysfunction (Chitwood, 2003), is increased.

In Experiment 2, the root invasion data in the presence of aldicarb were mirrored in the multiplication rate results, with lower Pf/Pi obtained from the AMF-inoculated plots compared with the noninoculated. The root invasion as well as the P content results should nevertheless be treated with caution because these assessments were only conducted once due to limited resources.

In the absence of aldicarb in Experiment 1, the significantly increased *G. pallida* hatch at wk-2 under AMF-inoculated plants relative to noninoculated plants, was not associated with a similar increase in Pf/Pi. On the other hand, the significant reduction in *G. pallida* multiplication in nematicide-treated plots inoculated with *G. intraradices* or *G. mosseae* compared with their noninoculated counterparts, illustrated that AMF application can be beneficial to the potato farmer as it seems to suppress the population size of *G. pallida*.

In a previous pot experiment where potatoes (cv. Golden Wonder) were pre-inoculated with AMF (using the same species-isolates as in this study) 2 wk before inoculation with *G. pallida* cysts, mycorrhization was found to have a significantly controlling effect on the nematode's reproduction not only in the presence but also in the absence of aldicarb. In the latter case, mycorrhization reduced Pf/Pi by 58% relative to that on noninoculated plants (Deliopoulos et al., 2008).

In both experiments, light microscopy studies of roots of 4-wk-old potato plants confirmed successful colonization by each of the three AMF. However, AMF root infection levels were lower in Experiment 1 (mean of 34.2%) than in Experiment 2 (47.5%), although this did not seem to have any influence on the *G. pallida* multiplication patterns. This variation could be attributed to differences in soil fertility levels between the two experiments; the soil in Experiment 1 had higher P content (101 ppm) than in Experiment 2 (40 ppm) and received N and K fertilizer in contrast with Experiment 2. This finding was in agreement with Habte and Manjunath (1987) who reported that mycorrhizal colonization is negatively correlated with P concentration in the soil solution. The fact that aldicarb application did not cause a negative effect on AMF colonization was in agreement with Vyas and Vyas (2000) in that carbamate nematicides do not have detrimental (but neither beneficial) effects on mycorrhizal fungi. In contrast, soil fumigants and fungicides applied as soil drenches have both been described to be detrimental to AMF (Menge, 1982; Perrin and Plenchette, 1993; Udaiyan et al., 1999). The absence of indigenous AMF in the roots of noninoculated plants was attributed to the suppression of native mycorrhizal populations during the growing of the preceding crop in the rotation. This was sugar beet in both trials, which is a non-mycorrhizal host as most members of the Chenopodiaceae (Brundrett, 2009).

Mycorrhization of potato roots had a slight enhancing effect on the concentration of P in the plant tissues of 6-wk-old plants, but only in the presence of aldicarb, which was another indication of the good compatibility between AMF and aldicarb. The reason for this positive interaction between these two components is likely to have been the combined result of capture of P by mycorrhizal hyphae in soil regions inaccessible to plant roots and reduced nematode root invasion, both associated with increased water and nutrient uptake (Hayman, 1983 and Grove et al., 1999, respectively).

Of the three AMF used, only inoculation with the two single isolates produced a weak, but not significant, increase (13% and 11% for *G. intraradices* and *G. mosseae*, respectively) in the total tuber yield of plants over noninoculated controls, in the aldicarb-treated plots of Experiment 1. This effect was mainly attributed to the significantly lower *G. pallida* multiplication observed on *G. intraradices*- and *G. mosseae*-inoculated plants as compared with noninoculated plants, in the presence of aldicarb.

The percentage AMF root length colonization did not seem to be closely related to yield response, because the three AMF exhibited uniform root colonization but the yields produced were similar in the absence and dissimilar in the presence of aldicarb. In the study of Deliopoulos et al. (2008), AMF colonization of the potato cv. Golden Wonder by Vaminoc was greater than by *G. intraradices* and *G. mosseae* but only the single AMF isolates increased total dry biomass (36%) and fresh tuber yield (22%) relative to noninoculated control plants. Further evidence for the lack of an association between AMF colonization and mycorrhizal dependency (MD, growth response of plant species to colonization by AMF; Plenchette et al., 1983) is provided by Pinochet et al. (1998) and by Okon et al. (2007). Therefore, the differential yield response of the potato plants to inoculation with the single and mixed AMF in the present study was not surprising given the evidence in the literature that plant species vary greatly in their dependency on AMF and consequently on the degree of benefit received (Pinochet et al., 1998; Van der Heijden et al., 1998; Koide, 2000; Deliopoulos et al., 2008; Jansa et al., 2008).

Recent advances in the areas of molecular genetics (real-time PCR) have demonstrated that single AMF present in multi-species mixtures compete with each other and certain species become predominant at the expense of the others (Jansa et al., 2008). The lower efficacy of Vaminoc compared with the single *Glomus* isolates on tuber yield may have also been the consequence of the single AMF being functionally redundant to each other (and/or with root hairs) in the Vaminoc mixture, but functionally complementary with the plant roots when used singly (Koide, 2000; Deliopoulos et al., 2008). This variation in mycorrhizal dependency of plant

species is very common in natural ecosystems and plays a major role in plant succession (Janos, 1980) and community structure (Van der Heijden et al., 1998). Overall, the results from this study and from previous research conducted by this research team (Ryan et al., 2003; Deliopoulos, 2004; Deliopoulos et al., 2008) have clearly shown that while the effects of mycorrhization on PCN life cycle components were consistent, the effects on plant growth and physiology parameters were more variable. A likely reason is that potato, as field research has demonstrated, is not a highly mycotrophic/AMF dependent plant, in contrast with other crops such as beans, corn or leek (Dalpé and Monreal, 2004).

The experiments described here showed that application of commercial AMF inocula at the time of potato planting had good compatibility with the nematicide aldicarb and significantly improved its performance against *G. pallida*, possibly via hatch stimulation during times of peak nematicide activity in the field. The enhancement in the effectiveness of aldicarb in two different commercial field sites following mycorrhization of potato plants was a promising outcome in the proposed use of AMF in integrated *G. pallida* control. However, further field experimentation will be needed to increase the confidence in these encouraging findings.

Other future challenges include assessing the efficacy of this model with other non-fumigant nematicides, such as oxamyl and fosthiazate, various potato cultivars and more AMF inocula. Validation of the beneficial effect of mycorrhization in such a variety of conditions coupled with the experience to be gained from such studies may lead to the commercial exploitation of AMF in potato production areas with known *G. pallida* problems.

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