Transfer and Development of *Pasteuria penetrans*

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Abstract: Pasteuria penetrans isolate P-20 has been attributed as the cause of soil suppressiveness to peanut root-knot nematode in Florida. In this study, *P. penetrans* was transferred from a suppressive site to a new site and established by growing susceptible hosts to the peanut root-knot nematode during both summer and winter seasons. When two soil fumigants, 1,3-dichloropropene (1,3-D) and chloropicrin, were applied broadcast at the rate of 168 liters/ha and 263 kg/ha, respectively, the bacterium was not adversely affected by 1,3-D but was adversely affected by chloropicrin. In autumn 2005, after the harvest of the second peanut crop, the greatest number of J2 was recorded in the chloropicrin-treated plots, followed by the non-fumigated plots and 1,3-D-fumigated plots. The percentage J2 encumbered with endospores, endospores per J2 and percentage of *P. penetrans*-infected females were greatest in the non-fumigated plots, followed by 1,3-D- and chloropicrin-fumigated plots. This study demonstrates that *P. penetrans* can be transferred from a suppressive site to a new site and increased to suppressive densities against the peanut root-knot nematode.

Key words: Arachis hypogaea, biological control, chloropicrin, 1,3-dichloropropene, fumigation, management, *Meloidogyne arenaria*, *Pasteuria penetrans*, peanut, peanut root-knot nematode, suppressive soil, transferability.

The peanut root-knot nematode *Meloidogyne arenaria* (Neal) Chitwood race 1 is a widespread and destructive nematode pathogen of peanut (Dickson and De Waele, 2005). It is estimated to infest 25% of the peanut acreage in the southeastern United States (Bridges et al., 1994) and is known as a dominant, virulent pathogen of peanut that causes substantial yield losses at relatively low population densities (Wheeler and Starr, 1987; McSorley et al., 1992). The current approach to manage the peanut root-knot nematode includes the integration of nematicides (both fumigants and non-fumigants), cultural practices and most recently the use of resistant cultivars (Kinloch, 2003; Dickson and De Waele, 2005; Rich and Kinloch, 2005; Anderson et al., 2006).

There are only a few examples of effective biological control of plant nematodes, and methodologies for demonstrating their effectiveness are still under development (Kerry, 1982; Stirling, 1991). *Pasteuria penetrans* (Thorne) Sayre & Starr is one agent identified that causes soil suppressiveness against root-knot nematodes (Stirling, 1984; Oostendorp et al., 1991; Chen et al., 1996; Weibelzahl-Fulton et al., 1996; Freitas et al., 2000; Timper et al., 2001). *Pasteuria penetrans* is an endospore-forming, gram-positive bacterium that is considered an important parasite of several of the agriculturally important *Meloidogyne* spp. Within this bacterial species exist strains that are specific to species within the genus *Meloidogyne* even down to the host race level of the nematode (Chen and Dickson, 1998). The bacterium is an obligate parasite of root-knot nematode, and so it has not been successfully cultivated on artificial media although numerous researchers have made such attempts (Chen and Dickson, 1998) and those efforts are currently being continued (Hewlett et al., 2004). Until an effective artificial medium is found, *P. penetrans* must be cultivated on nematodes grown on a host crop in the greenhouse (Stirling and Wachtel, 1980) or in the field.

Although the organism shows great promise as a soil suppressive agent, no studies have been carried out to determine whether it can be transferred from a suppressive site and established and developed in a new field site. Commonly used nematicides including the soil fumigant 1,3-D are not known to be detrimental to *P. penetrans* (Chen and Dickson, 1998); however, fumigants containing chloropicrin are known to be detrimental to the bacterium (Frietas et al., 2000). The objectives of this study were to investigate whether *P. penetrans* can be transferred from a suppressive site to a new site and increased to suppressive levels against the peanut root-knot nematode, *M. arenaria* race 1. It is not possible to maintain non-*P. penetrans*-infested plots, thus we included in this investigation treatments with chloropicrin, which reduces incidence of the bacterium, and with 1,3-D, which reduces incidence of the peanut root-knot nematode.

Materials and Methods

A 25 m × 185 m field located at the Plant Science Research and Extension Center, Citra, FL, was prepared for infestation with *P. penetrans* and *M. arenaria*. The field was deep ploughed and disked before inocula were added. The soil was classified as an Arredondo fine sand (92.7% sand, 3.9% silt, 3.4% clay; <1% organic matter; pH 5.5). The site was chosen because pre-treatment sampling revealed no detectable root-knot nematodes or *Pasteuria* spp. present.

Transfer and establishment of P. penetrans and M. arenaria: Pasteuria penetrans isolate P-20 (Oostendorp et al., 1990) was collected twice from a *M. arenaria* suppressive site in Levy Co., FL (Dickson et al., 1994; Cetintas and Dickson, 2004, 2005). The bacterium was transferred in the form of endospore-filled female root-knot nematode cadavers inside dried peanut roots, pegs and pods,

and okra roots. The first (peanut, *Arachis hypogaea* L. cv. Georgia Green) and second (okra, *Abelmoschus esculentus* L. Moench cv. Clemson Spineless) transfers were in autumns of 2002 and 2003, respectively. Both crops were grown on 2.6 ha before they were dug and left on the soil surface to dry for 2 wk. The dried crop material was then collected and broadcast spread by hand over the freshly prepared field site. The dried material was incorporated into the soil approximately 10-cm deep with a rototiller.

Meloidogyne arenaria race 1 was introduced at the new field site on 21 March and again on 18 September 2003. The field was divided into 54 plots, each consisting of four rows 15-m long by 91-cm wide with 45-cm row spacing. Plots were established on 1.8 m centers, and a cultivating sweep was used to open furrows 20-cm deep in the center of the wheel tracks. The first introduction of *M. arenaria* was from infested soil and galled tomato roots. This inoculum, which was equally distributed in the open furrows and covered, was grown on tomato (*Lycopersicum esculentum* Mill. cv. Rutgers) in 500 15-cm-diam. pots. The inoculum for the second introduction was obtained by collecting *M. arenaria*-galled peanut roots, pods and pegs from a grower field in Levy Co., FL. Four coolers (30-cm deep × 30-cm wide × 52-cm long) were filled with heavily galled root, pods and pegs for transport and distribution into the 20-cm deep furrows made in the center of each of the beds. Before this inoculum was collected, the nematode's identity was confirmed based on morphology and isozyme phenotypes of females extracted from roots, pegs and pods (Esbenshade and Triantaphyllou, 1985). Thirty females were randomly selected and crushed on a glass slide, covered with a cover slip, and examined at ×400 magnification for the presence or absence of *P. penetrans* endospores. No *P. penetrans* endospores were detected from females extracted from roots or J2 extracted from soil.

No attempt was made to quantify the amount of *P. penetrans* or *M. arenaria* inoculum transferred because of the extremely high variability of root-knot nematode females, egg masses and *P. penetrans* endospore-filled females contained within roots, pods and pegs. We considered the density of endospores attached to J2 and number of J2 extracted from the newly infested field as an initial base-line infestation level for both *P. penetrans* and *M. arenaria*.

Pasteuria penetrans development and assays: Peanut cultivar Georgia Green was seeded into each plot on 16 April 2003, followed by winter wheat (*Triticum aestivum* L. cv. AGS 2000) on 2 October 2003. These crops were grown to allow both *M. arenaria* and *P. penetrans* to become established and to increase in population densities. Production practices for growing peanut and wheat in the area were followed (Mask et al., 1992; Whitty, 2002). The peanut and wheat crops were ploughed under on 9 September 2003 and 21 April

2004, respectively. Soil samples were collected before planting and at harvest of each crop. Six cores (2.5-cm-diam., 20-cm deep) of soil were taken per plot with a cone-shaped sampling tube. The soil from each plot was mixed thoroughly, and nematodes were extracted from 100 cm^3 of soil by centrifugal-flotation method (Jenkins, 1964). Six plants were chosen randomly from each plot at harvest to determine root-knot nematode galling based on a scale of 0 to 5 (Taylor and Sasser, 1978).

A soil bioassay was conducted at harvest from each crop to determine the presence of *P. penetrans* in soil. For the bioassay, soil taken from each plot was air-dried, and 40 g was placed in a 50-ml polyethylene centrifuge tube. Soil water content was adjusted to 100% (saturated) capacity to increase the rate of endospore attachment to J2 (Brown and Smart, 1984). Then, 500 1- to 3-d-old *M. arenaria* J2 were added, and the tubes were left uncovered at room temperature. Three days later, the J2 were extracted from the soil using the centrifugal-flotation method (Jenkins, 1964). The number of endospores attached per J2 was made by observation with an inverted microscope at ×200 magnification.

Galled roots from six peanut plants were collected randomly from each plot at harvest. Fresh roots, pods and pegs from each sample were incubated for 2 to 3 d at room temperature in 10% Rapidase Pomaliq 2F (Gist-Brocades Pomaliq product number 7003-A/DSM, Food Specialties, Menominee, WI), 50 mM NaOAc (pH 5.0), and 0.1% $CaCl_2$ at approximately 50:50 v/v (Charnecki, 1997). Twenty milliliters of the solution was used per 4 g of the root, pod and peg mixture. The samples were placed on a sieve with 600-µm aperture (30 mesh) nested over a sieve with 150-µm aperture (100 mesh) and sprayed with a heavy stream of tap water (Hussey, 1971). Thirty females were randomly selected and crushed on a glass slide, covered with a cover slip, and examined at ×400 magnification for the presence or absence of *P. penetrans* endospores.

Fumigation: In spring 2004, following the wheat crop, the field was arranged in a randomized complete block design with three treatments replicated 18 times. Each plot measured 6.1-m long × 3.6-m wide, with a 1.8-m wide non-tilled border separating each plot. The three treatments were: (i) non-fumigated, (ii) chloropicrin at the rate of 263 kg/ha, and (iii) 1,3-D at the rate of 168 liters/ha. All plots were infested with *P. penetrans* and *M. arenaria* race 1. The fumigants were applied broadcast with six parachisels (Riegel et al., 2000), each spaced 30-cm apart and adjusted to deliver the fumigants 20-cm deep beneath the final soil surface. The soil surface was sealed and compacted using a heavy roller to prevent premature loss of the fumigants to the atmosphere.

Two weeks following fumigation, peanut cultivar Georgia Green was seeded in all plots. In summer 2004, due to the effect of two hurricanes, six replicates were

lost because of prolonged flooding. The peanut crop was dug 7 October 2004. Three weeks later a winter cover crop, common vetch (*Vicia sativa* L.), was seeded. The vetch roots were dug 8 April 2005. Data collected following each crop included J2 per 100 cm³ of soil, endospores per J2, percentage infected females, root and pod gall indices for peanut, and root galling and egg mass indices for vetch.

In spring 2005, following the winter vetch crop, the fumigant treatments were again applied in the same manner and plots as before in 2004. Peanut cultivar Georgia Green was seeded on 10 May 2005. At the end of the peanut crop, data were collected as described above. In addition, subjective plant growth ratings and peanut yield data were collected per plot. The peanut growth ratings were scored on a subjective scale of 1 to 10 (1 = poor growth and 10 = good growth) (Cetintas and Dickson, 2004).

Statistical analysis: Data were subjected to analysis of variance (ANOVA) with SAS software (SAS Institute, Cary, NC), and mean treatment differences were separated and compared using Waller-Duncan k ratio ($k = 100$) t-test. Microsoft Excel (Microsoft, Redmond, WA) was used for regression analysis. Data for number of J2 per 100 cm³ of soil and endospore per J2 were transformed using $\log_{10} (x + 1)$ before statistical analysis. Untransformed numbers are presented in the text and table.

Results

Transfer and establishment of M. arenaria and P. penetrans: In spring 2003 the soil bioassay showed the level of *P. penetrans* endospores per J2 after introduction was

1.3 ± 0.7 endospores/J2. In the autumn, following the first peanut crop, the number of endospores per J2 decreased slightly to a mean of 1.1 ± 1.0. However, following the winter wheat crop, spring 2004, the mean number of endospores per J2 had increased to 5.0 ± 2.2. The mean J2 per 100 cm³ in spring 2004 following wheat harvest was 10.4 ± 0.8. These means were based on the overall means of all plots. No data on proportions of J2 with endospores attached were collected at this time.

Fumigant effects: In autumn 2004, after the peanut harvest, there were differences between non-fumigated plots and the fumigant-treated plots with respect to the number of J2 per 100 cm³ of soil, endospores per J2, percentage infected females, and root and pod-gall indices ($P \le 0.05$). The greatest number of J2 were found in the chloropicrin-treated plots, an intermediate number in the non-fumigated plots, and the smallest number in the 1,3-D-treated plots ($P \le 0.05$) (Table 1). The non-fumigated plots had greater numbers of endospore-encumbered J2 than the chloropicrin- and 1,3-D-treated plots ($P \le 0.05$). The largest percentage of *P. penetrans*-infected females was observed in non-fumigated plots, followed by 1,3-D- with an intermediate number, and the smallest number in chloropicrin-treated plots ($P \le 0.05$). The root and pod-gall indices were highest in non-fumigated plots and lowest in 1,3-D-treated plots ($P \le 0.05$).

In spring 2005, after the vetch harvest, there was a trend similar to that observed in autumn 2004 with the exception that root galling was greater in the chloropicrin-treated plots as compared with non-fumigated and 1,3-D-treated plots ($P \le 0.05$) (Table 1). Egg mass indices were similar to galling indices. In autumn 2005,

TABLE 1. Effect of chloropicrin and 1,3-D on *Pasteuria penetrans* and *Meloidogyne arenaria* race 1 following two seasons of peanut, 2004 and 2005, and a winter cover crop of vetch 2004–05. All plots were infested with *P. penetrans* and *M. arenaria*, including the non-fumigated.

Treatment	Parameters[a]					
	J2/100 cm³	Endo/J2[b]	%IF[c]	Root GI[d]	POD GI[d]	EgM
Autumn 2004 (at peanut harvest)						
Non-fumigated	84.7 b	7.4 a	49.0 a	2.6 a	2.6 a	
Chloropicrin	144.8 a	2.4 b	18.8 c	1.8 b	1.9 ab	
1,3-D	30.2 c	2.4 b	32.6 b	1.1 c	1.6 b	
Spring 2005 (at vetch harvest)						
Non-fumigated	16.8 c	2.0 a	26.7 a	1.5 c	—	1.2 c
Chloropicrin	79.7 a	0.7 b	4.1 b	3.7 a	—	4.1 a
1,3-D	39.0 b	1.2 b	7.5 b	2.6 b	—	3.0 b
Autumn 2005 (at peanut harvest)						
Non-fumigated	63.6 b	3.6 a	54.9 a	1.1 b	1.1 b	
Chloropicrin	152.1 a	0.5 b	22.1 c	4.2 a	4.1 a	
1,3-D	51.6 b	1.4 b	47.4 b	1.4 b	1.3 b	

[a] Values are means of 12 replicates; means within a column followed by the same letter(s) are not significantly different according to Waller-Duncan k ratio ($k = 100$) t-test ($P \le 0.05$). Data for J2 per 100 cm³ of soil and endospore per J2 were transformed using $\log_{10}(x + 1)$ before statistical analysis. Untransformed numbers are presented.

[b] The first 20 second-stage juveniles (J2) were observed for the presence of endospores attached to the nematode cuticle.

[c] Percentage infected females was based on the first 30 females observed for the presence or absence of endospores inside their body.

[d] Root gall, pod and egg mass (EgM) indices were rated using a 0–5 scale where 0 = no galls, 1 = 1–2 galls, 2 = 3–10 galls, 3 = 11–30 galls, 4 = 31–100 galls, 5 = more than 100 galls (Taylor and Sasser, 1978).
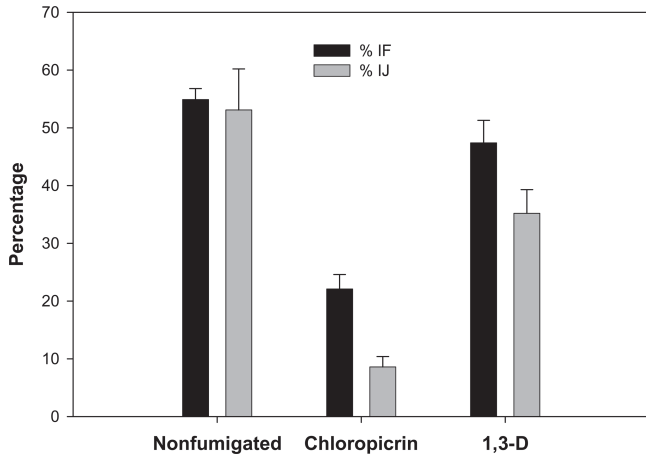
FIG. 1. Effect of chloropicrin and 1,3-D on percentage of *Pasteuria penetrans*-infected *Meloidogyne arenaria* race 1 females (% IF) and second-stage juveniles (J2) with endospores attached (% IJ) at harvest September 2005. All plots were infested with *P. penetrans* and *M. arenaria* including the non-fumigated plots.

there were no differences between the number of galls in non-fumigated and 1,3-D-treated plots ($P \leq 0.05$) (Table 1). The number of endospores per J2 was greatest in the non-fumigated plots with no differences between 1,3-D- and chloropicrin-treated plots ($P \leq 0.05$). There were consistently greater numbers of J2 per 100 cm^3 of soil in the chloropicrin-treated plots on peanut roots during each of the two seasons of growing peanut and one season of vetch cover crop ($P \leq 0.05$) (Table 1).

The percentage of J2 extracted from the field soil with endospores attached (based on the bioassay of field soil) and the proportion of infected females were largest in the non-fumigated plots followed by 1,3-D-treated plots, and least in chloropicrin-treated plots, with all three being different ($P \leq 0.05$) (Fig. 1). The plant growth indices of the peanut crop in the field were low in chloropicrin-treated plots but greater in
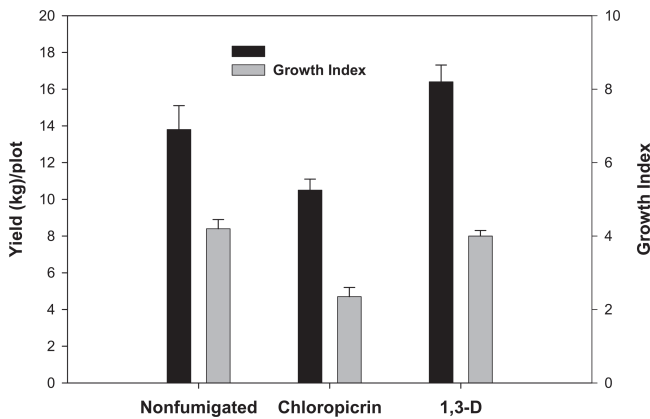

FIG. 2. Effect of chloropicrin and 1,3-D on plant growth and yield (kg) per plot of peanut grown in a site infested with both *Pasteuria penetrans* and *Meloidogyne arenaria* race 1, at harvest September 2005. Plant growth index rated on a scale of 1 - 10 (1 = poor growth, 10 = good growth). All plots were infested with *P. penetrans* and *M. arenaria* including the non-fumigated plots.

both the non-fumigated plots and the 1,3-D-treated plots. The latter two were not different ($P \leq 0.05$) (Fig. 2). The non-fumigated plots and 1,3-D-treated plots had the greatest peanut yield per plot ($P \leq 0.05$) compared with chloropicrin-treated plots ($P \leq 0.05$).

A linear regression model Y = 0.958× + 29.001, r^2 = 0.0132, P = 0.5046 of the relationship between endospore numbers per J2 in spring before planting peanut and the proportion of infected females at harvest in autumn 2004 was not significant ($P \leq 0.05$) (Fig. 3A). However, in autumn 2005, a regression model, $\underline{Y}$ = 12.683Ln($\underline{x}$) + 26.773, r^2 = 0.2499, P = 0.0019, demonstrated a significant relationship between endospore numbers per J2 in spring before a peanut crop to percentage infected females in autumn at harvest ($P \leq 0.05$) (Fig. 3B).

DISCUSSION

We demonstrated that *P. penetrans*-infested *M. arenaria* cadavers in dried crop roots can be transferred from one field site to another and the bacterium increased to suppressive levels against the peanut root-knot nematode. Only a few nematode-suppressive field
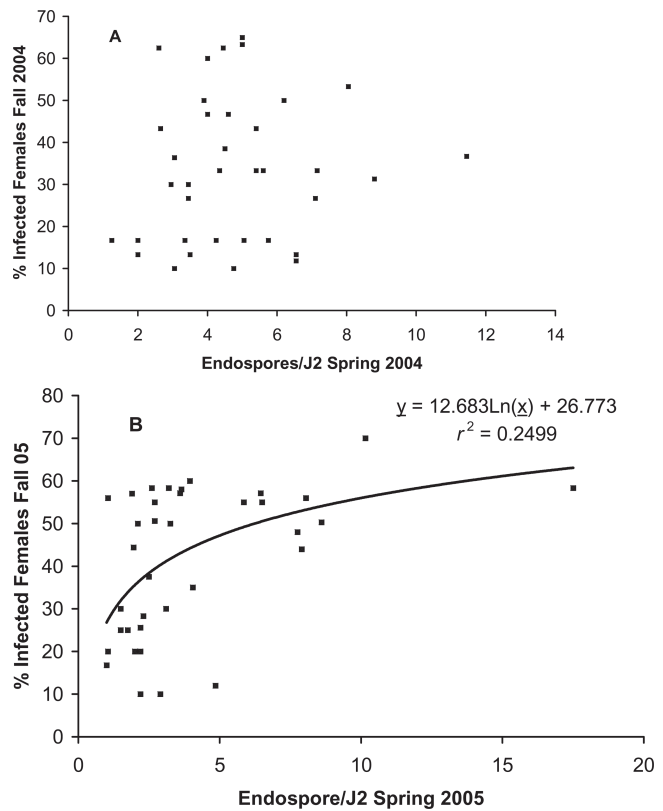

FIG. 3. Effect of *Pasteuria penetrans* endospore density in soil in spring before a peanut crop on the percentage infected females in fall after peanut harvest. A) Correlation of endospores per J2 in soil before planting spring 2004 to percentage *P. penetrans*-infected females at harvest in autumn 2004 was not significant ($P \leq 0.05$). B) Regression of endospore per J2 before planting a peanut crop in spring 2005 to percentage infected females after the harvest of the peanut crop in autumn 2005 ($P \leq 0.05$).

sites have been documented (Kerry, 1988; Kluepfel et al., 1993; Dickson et al., 1994; Westphal and Becker, 1999; Pyrowolakis et al., 2002; Westphal, 2005). Amendment of steam-sterilized greenhouse soil with *P. penetrans*-infested soil resulted in suppression of *Meloidogyne incognita* (Mankau, 1975). Biological suppressiveness against *Heterodera schachtii* was transferable with small portions of soil under both field and greenhouse conditions (Westphal and Becker, 1999). Transferability of soil suppressiveness is an indication of specific soil suppressiveness against plant-parasitic nematodes (Westphal, 2005).

In a situation where crop residue is used as a conveyance of *P. penetrans*, special care must be taken to ensure that the residue is sufficiently dry to avoid transferring live nematodes and other live disease-inducing propagules alongside the *P. penetrans* propagules. In our case, there was no rainfall during the two-week drying periods, which greatly facilitated the drying of the plant material lying on the soil surface. It was expected that *P. penetrans* endospores would survive the relatively high surface soil temperatures such as those that occur in Florida during the early autumn months, whereas nematodes in the exposed roots would not. Others have reported that endospores of *P. penetrans* resist various chemicals and stressful environmental conditions (Mani, 1988; Williams et al., 1989). Endospores of *P. penetrans* were reportedly killed by autoclaving but only slightly affected by microwaving (Chen et al., 1995; Weibelzahl-Fulton, 1998). We knew the site of origin for *P. penetrans* was infested with a mixed population of *M. arenaria* and *M. javanica* (Cetintas, et al., 2003; Cetintas and Dickson, 2004), thus there was concern that we might accidentally transfer some live *M. javanica* juveniles or eggs to the new site. Over the course of four years at the new site, we examined the electrophoretically derived enzyme profiles of over 200 females collected arbitrarily from galled peanut roots without detection of any patterns other than *M. arenaria*. Also, the site of origin was known to be heavily infested with propagules of *Sclerotium rolfsii*, but again this disease agent never materialized at the new site after the dried plant material was transferred.

After the transfer of *P. penetrans* and subsequent introduction of *M. arenaria*, both the hyperparasite and nematode pathogen amplified rapidly. Surprisingly, there were no differences between endospores per J2 in soil before and immediately following the peanut harvest during the first year. In fact, endospore density in soil was lowest at this time. However, six months later following a wheat cover crop, the endospore density in the soil increased exponentially. A possible explanation is that most of the endospores were still held within the female cadavers that resided within peanut roots at harvest when samples were taken. It is only after those roots decompose that the female cadavers

release their endospore burden. This most likely occurs over the winter months. Hence, to correctly estimate endospore densities in soil following an autumn crop, the best time to sample may be in early spring. This is a departure from traditional methology where nematode and endospore densities in soil are usually estimated immediately following a summer or autumn crop.

The wheat winter cover crop planted in autumn of 2003 had very few discernable galls. The wheat cultivar was tested as a host for *M. arenaria* race 1 in the greenhouse and proved to be a poor host. Even if the winter crop were a highly susceptible host to *M. arenaria* race 1, large numbers of endospores in soil would likely attach to the juveniles and hinder their mobility and subsequent infection of the wheat roots. Reductions in plant infectivity have been observed when nematodes are encumbered with 15 to 20 endospores (Brown and Smart, 1985), but just recently we demonstrated that as few as 3 endospores/juvenile interfered with J2 penetrating tomato roots (Kariuki et al., 2006).

The effective role of 1,3-D as a soil fumigant against *M. arenaria* was evident, resulting in the lowest number of J2 per 100 cm^3 of soil being detected in 2004 and 2005 after peanut crop. However, after the vetch crop, nematode population densities in all plots declined and were lowest in the non-fumigated plots. The differences in *P. penetrans* population densities in 1,3-D plots and the non-fumigated plots could be attributed to the fact that when 1,3-D was applied, a high percentage of J2 in the 0- to 20-cm depth were killed, thus limiting development of *P. penetrans*. Infection that occurred later could have resulted from re-infestation of J2 from below the fumigated soil or from nematode eggs that had not hatched in time to be affected by the fumigant. Prot (1978) reported that *Meloidogyne* spp. J2 have the ability to migrate vertically up to 50 cm. This movement can be attributed to thermal gradients (Diez and Dusenbery, 1989). Computer modeling studies suggest that the thermal dynamics in the soil environment would cause the nematodes to move toward the soil surface (Dusenbery, 1988). Such movement would render J2 available in the root rhizosphere where the majority of endospores are concentrated. Juveniles encumbered with *P. penetrans* endospores are needed in order for further reproduction of the bacterium.

Following harvest of the second peanut crop, autumn 2005, there was a strong indication of soil suppressiveness of *M. arenaria* in the non-fumigated plots as compared with the chloropicrin-treated plots. Chloropicrin reduced the incidence of *P. penetrans*, and this confirms earlier reports that the compound is detrimental to *P. penetrans*, not necessarily for the attachment process, but for the continued development of the bacterium within the female nematodes (Freitas et al., 2000). It should be pointed out that the exact mode of action of

chloropicrin on *P. penetrans* is unknown. However, where 100% chloropicrin or 33% chloropicrin is applied under polyethylene mulch in tomato culture, no endospore-filled root-knot nematode females were detected (Freitas et al., 2000). We observe this same phenomenon in grower fields fumigated with methyl bromide-chloropicrin mixtures in Florida.

The largest percentage of *P. penetrans*-infected females in non-fumigated plots differed from 1,3-D- and chloropicrin-treated plots. In terms of yield, there were no significant differences between 1,3-D-treated plots and non-fumigated plots. The degree of control of *M. arenaria* in the non-fumigated plots (infested with both *P. penetrans* and *M. arenaria*) was similar to that of the 1,3-D-treated plots. Cultivation of a susceptible host crop for more than one season is needed for *P. penetrans* to build up densities to suppressive levels (Melki et al., 1998).

Attempts were made to predict the level of suppressiveness using percentage infected females based on endospores per J2 in the soil before planting using a regression model. The regression model was not significant during the second year following peanut, but the model was significant after the third year following peanut. The use of a soil bioassay to determine the number of endospores per J2, which involves drying 40 g of soil to kill all the nematodes and then re-introducing J2, gives better detection of *P. penetrans* than extracting endospore-encumbered J2 from soil (Cetintas and Dickson, 2004). Detecting endospore densities in the soil with precision before planting a crop or early in the growing season may help growers know whether or not to apply other management practices, especially when the crop being planted is susceptible to the nematode in question. In this particular study, the correlation between endospores per J2 before the peanut crop was planted and the percentage infected females at peanut harvest was only evident after the third peanut crop, and even then it was relatively low ($r^2 = 0.2499$). This may be evidence that there are many other factors that affect endospore densities in soil and their subsequent attachment and infection of nematode hosts (Chen and Dickson, 1998; Cetintas and Dickson, 2005).

In summary, *P. penetrans* can be transferred from one site to another and become established once a nematode host and a crop susceptible to the nematode host are introduced. *Pasteuria penetrans* is not affected adversely by application of the soil fumigant 1,3-D; however, chloropicrin apparently interferes with *P. penetrans* development in root-knot nematode females. Thus, chloropicrin is a useful tool in the demonstration of the role of *P. penetrans* in soil suppressiveness. Potentially, 1,3-D could reduce the risk of yield loss in *M. arenaria*-infested fields when population densities of *P. penetrans* are too low to suppress the nematode.

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