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Persistence and Suppressiveness of *Pasteuria penetrans* to *Meloidogyne arenaria* Race 1[1]

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Abstract: The long-term persistence and suppressiveness of *Pasteuria penetrans* against *Meloidogyne arenaria* race 1 were investigated in a formerly root-knot nematode suppressive site following 9 years of continuous cultivation of three treatments and 4 years of continuous peanut. The three treatments were two *M. arenaria* race 1 nonhost crops, bahiagrass (*Paspalum notatum* cv. Pensacola var. Tifton 9), rhizomal peanut (*Arachis glabrata* cv. Florigraze), and weed fallow. Two root-knot nematode susceptible weeds commonly observed in weed fallow plots were hairy indigo (*Indigofera hirsuta*) and alyce clover (*Alysicarpus vaginalis*). The percentage of J2 with endospores attached reached the highest level of 87% in 2000 in weed fallow, and 63% and 53% in 2002 in bahiagrass and rhizomal peanut, respectively. The percentage of endospore-filled females extracted from peanut roots grown in weed fallow plots increased from nondetectable in 1999 to 56% in 2002, whereas the percentages in bahiagrass and rhizomal peanut plots were 41% and 16%, respectively. Over 4 years, however, there was no strong evidence that endospores densities reached suppressive levels because peanut roots, pods, and pegs were heavily galled, and yields were suppressed. This might be attributed to the discovery of *M. javanica* infecting peanut in this field in early autumn 2001. A laboratory test confirmed that although the *P. penetrans* isolate specific to *M. arenaria* attached to *M. javanica* J2, no development occurred. In summary, *P. penetrans* increased on *M. arenaria* over a 4-year period, but apparently because of infection of *M. javanica* on peanut at the field site root-knot disease was not suppressed. This was confirmed by a suppressive soil test that showed a higher level of soil suppressiveness than occurred in the field ($P \leq 0.01$).

Key words: Arachis hypogaea, biological control, *Meloidogyne arenaria, M. javanica,* nematode, *Pasteuria penetrans,* peanut, persistence, root-knot nematode, suppressive soil.

Pasteuria penetrans (Thorne) Sayre & Starr is an obligate, endospore-forming bacterium that has been shown to effectively suppress root-knot nematode populations in field and microplots experiments (Brown and Smart, 1985; Chen et al., 1996; Freitas et al., 2000; Oostendorp et al., 1991; Stirling 1984; Weibelzahl-Fulton et al., 1996). The organism's extended survival in soil, host specificity, tolerance to heat, desiccation, and some nematicides make *P. penetrans* one of the more promising biological control agents for plant-parasitic nematodes (Bekal et al., 2000; Chen and Dickson, 1998; Stirling, 1991).

Pasteuria penetrans was reported (Dickson et al., 1994) to cause suppressiveness of *Meloidogyne arenaria* race 1 in a peanut field located near Williston, Levy County, Florida. This site had been used for peanut nematode research for several years beginning in 1970. Around the mid-1980s, the site began to show signs that the root-knot disease problem was declining. Gradually, over a period of several years, peanut yield increased and root-knot nematode galling on roots, pegs, and pods decreased greatly (Dickson et al., 1991, 1994). Examination of second-stage juveniles (J2) extracted from soil samples taken across the field showed that 32% of them had large numbers of endospores of *P. penetrans* attached. In 1990, the entire 2-ha site was planted with peanut and the yield averaged 5,605 kg/ha, which was above the county average for that year. The conclusion was that the site had become highly suppressive to the peanut root-knot nematode and *P. penetrans* had built to a highly suppressive density.

In most studies, suppressiveness of soils to *Meloidogyne* spp. was observed after several years of crop production (Bird and Brisbane 1988; Chen et al., 1994; Mankau, 1980; Minton and Sayre, 1989). There are few or no data on the long-term persistence of *P. penetrans* in suppressive sites and whether or not suppressiveness can be maintained for a long period of time. Also, no data exist on the effect of growing various crops or nonhosts on the nematode, the bacterium, or the suppressiveness of soils. These become important considerations if we are to understand soil suppressiveness to plant-pathogenic nematodes and acquire knowledge of factors that maintain suppressiveness, including the effects of growing different plant species. In early autumn 2001, *M. javanica,* which is not a host for *P. penetrans* isolate P-20, was surprisingly discovered infecting peanut in this field (Cetintas et al., 2003). Its detection conflicted with our objective to elucidate the role of *P. penetrans* in root-knot disease suppression in this field. This study was designed to determine the long-term effects (9 years) of two established crops, bahiagrass and rhizomal peanut, and weed fallow followed by 4 years of continuous peanut on persistence of *M. arenaria* and *P. penetrans.*

Materials and Methods

Persistence and suppressiveness in field soil: A field plot experiment with a randomized complete block design was established with three treatments and replicated 10 times in 1991. The three treatments were two *M. arenaria* race 1 nonhost crops (bahiagrass *Paspalum notatum* cv. Pensacola var. Tifton 9), rhizomal peanut (*Arachis glabrata* cv. Florigraze), and weed fallow. Two root-

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knot nematode-host weeds observed in the weed fallow plots were hairy indigo (*Indigofera hirsuta* l.) and alyce clover [(*Alysicarpus vaginalis* (l)]. The weed fallow plots were planted with rye (*Secale cereale* l. cv. Wrens Abruzzi) during the autumn-winter season as a cover crop. Each plot was 38 m long and 10.6 m wide, with a 2.4-m-wide non-tilled border separating each plot. The soil was classified as Arredondo fine sand (92.5% sand, 4% silt, 3.5% clay, and <1% organic matter; pH 7.2). From 1991 to 1999, the site received minimum inputs. No fertilizer, irrigation, or pest-pathogen management tactics were applied; however, once or twice each year weeds were mowed in the weed fallow plots and in all borders and alleyways. Bahiagrass established well during the first two growing seasons, but rhizomal peanut required about 3 years to give complete ground cover.

Development of M. arenaria and P. penetrans: Experiments on the population densities, persistence, and suppressiveness of *M. arenaria* and *P. penetrans* were begun in 1999 and continued through 2002. Peanut was planted after bahiagrass and weed fallow in plots for 4 consecutive years (1999 to 2002) and in the rhizomal peanut plots for 3 consecutive years (2000 to 2002). In 1999, the bahiagrass and weed fallow plots were prepared for planting peanut by mowing, plowing, and disking. The rhizomal peanut plots could not be plowed or disked because of a deep and thick thatch layer. Therefore, glyphosate herbicide was sprayed over the rhizomal peanut plots in summer 1999 to kill the plants, and the plots were prepared for planting to peanut in 2000. Peanut cv. Florunner was planted in 1999, cv. Southern Runner in 2000, and cv. Georgia Green in 2001 and 2002. Each plot consisted of 10 rows planted on 90-cm row spacing. Production practices common for growing peanut in the area were applied (Whitty, 2002).

Twelve cores (2.5-cm-diam., 20 cm deep) of soil were taken from each plot each growing season with a cone-shaped sampling tube 1 to 3 days before planting (Pi), near mid-season [55–65 days after planting (Pm)], and at crop harvest (Pf). Soil cores from each plot were combined, mixed thoroughly, and nematodes were extracted from a 100-cm^3 subsample by a centrifugal-flotation method (Jenkins, 1964). All plant-parasitic nematodes were counted. The number of *P. penetrans* endospores attached to the first 20 J2 observed from Pi (spring) and Pf (autumn) extractions were counted. Plant growth ratings were determined at or near harvest based on a scale of 1 to 10 (1 = poor growth and 10 = good growth). At the end of each season peanut plants were dug, left on the soil surface for 1 or 2 days to dry, and harvested with a peanut combine. Twenty-six plants were chosen randomly from each plot at harvest every season to determine root-knot nematode galling based on a scale of 0 to 100 (0 = no galls on roots, 10 = 10%, and 100 = 100% of the root system galled) (Barker et al., 1986). Yield data were recorded from the middle six rows (total of 10 rows in a plot) of each plot. Although all peanut plants were dug in 2002, yield was not determined because plants in all plots were severely stunted from drought during the first 2 months of the growing season.

Soil bioassay for P. penetrans: A soil bioassay was conducted preplant and at harvest to determine the presence of *P. penetrans* in the soil. A portion of soil from the soil samples taken from each plot (as described in previous paragraph) was air-dried and 40 g from each sample was placed in a 50-ml sterile polyethylene centrifuge tube. Soil water content was adjusted to 100% capacity (saturated) to increase the rate of endospore attachment to J2 (Brown and Smart, 1984). Then 500, 1- to 3-day-old J2 of *M. arenaria* were added and the tubes left uncovered at room temperature. Three days later the J2 were extracted by a centrifugal-flotation method (Jenkins, 1964). The following scale was used to estimate the number of endospores attached per J2: 0 = none, 1 = 1–2, 2 = 3–5, 3 = 6–15, 4 = 16–100, 5 = > 100. Observations were made with an inverted microscope, and data were recorded from the first 20 J2 observed per sample.

Number of females of M. arenaria infected with P. penetrans: Galled roots from 26 peanut plants were collected arbitrarily from each plot at harvest and kept in a paper bag until they were processed. After 5 to 6 weeks, about 10 g of dry roots from each sample were incubated for 2 days in an enzymatic maceration solution. The solution consisted of 50 ml of 0.5 M sodium acetate (pH 5.0), 50 ml of Pomaliq 2 F (Gist-Brocades, Charlotte, NC), 500 µl of 1.0 M calcium chloride, 400 ml deionized water (Charnecki, 1997). The samples were washed vigorously over stacked-sieves with 600-µm-pore openings (top) and 75-µm-pore openings (bottom). Forty females were selected arbitrarily from the females collected on the bottom sieve. These females were crushed on a glass slide, covered with a cover slip, and examined microscopically for the presence of *P. penetrans* endospores.

Suppressive soil test: At the conclusion of this experiment 120 liters of soil was collected from each of three plots (bahiagrass, rhizomal peanut, and weed fallow) by taking 20 soil cores (2.5 cm × 20 cm deep) arbitrarily from each plot. The soil from each plot was mixed thoroughly and divided into four sub-samples of 30 liters each for the four treatments that were to be imposed. The treatments were autoclaving, microwaving, air-drying, and untreated. Each treatment was replicated five times. Moisture of the soil was 18% when prepared for treatment.

Autoclaving: Autoclaving was used to kill all biota in the soil (Chen et al., 1995; Weibelzahl-Fulton et al., 1996), thereby allowing root-knot nematodes to be infective. Previously sterilized clay pots (15-cm-diam.) were filled with about 1 liter of soil, and the pots were covered with aluminum foil and autoclaved for 99 min-

utes at 55 kPa. After the pots cooled to room temperature they were autoclaved again (Mulder, 1979).

Microwaving: Treating soil by microwaving was used as an improved method for reducing possible fungal antagonists. Earlier observations have shown that microwaving soil is an effective method to reduce fungal antagonists (Chen et al., 1995; Ferris, 1984; Weibelzahl-Fulton et al., 1996). The 30 liters of soil were microwaved by placing 1 kg of soil at a time in even layers in plastic bags, each left open, and treated for 3 minutes (Ferris, 1984) at full power (625 W, 2,450 MHz). The soil temperature rose to about 75 °C.

Air-drying and untreated: Air-dried and untreated soils would contain any natural predators or parasites that may be suppressive to *M. arenaria*. For air-drying, the soil was placed in 4- to 5-cm-thick layers on a polyethylene sheet on a greenhouse bench for 2 weeks. The soil was stored in a greenhouse until used. The untreated soil was stored at 10 °C until used.

Following treatment, 100 ml of water was added to the air-dried soil and 50 ml was added to all other soils. Then, 2,500 J2 of *M. arenaria* were dispensed in 8 ml of water to each of four holes (2 ml of nematode suspension each) about 5 cm deep in each pot. After 1 week, one 4-week-old tomato seedling, *Lycopersicon esculentum* Mill. cv. Rutgers, was transplanted into each pot. The pots were kept in a growth room at 28 °C to 32 °C and in 16 hours of light. Every 10 to 14 days insects were managed by using insecticidal soap, and every week the plants were fertilized by Peters Professional 20-20-20 (N, P_2O_5, and K_2O, respectively) (Scotts-Sierra Horticultural Products, Maryville, OH) at 3 g/liter of water. After 57 days the plant stems were cut off at ground level and discarded. The plant roots were removed, washed free of soil, and stored in plastic bags at 10 °C for processing. The roots were examined over a 2-day period and the number of root galls counted. Eggs were counted following extraction by the NaOCl method (Hussey and Barker, 1973).

Origin of the root-knot nematode isolate: The isolate of *M. arenaria* race 1 used in the bioassay and the suppressive soil test originated from peanut grown at the former University of Florida Green Acres Agronomy Farm, Alachua County, Florida. Eggs of the nematode, which was cultured on tomato (*Lycopersicon esculentum* cv. Rutgers), were collected from galled roots by treating them with 0.5% sodium hypochlorite (Hussey and Barker, 1973). Eggs and J2 were caught on a sieve with 25-µm-pore openings. The eggs were hatched on a modified Baermann funnel (Rodríguez-Kábana and Pope, 1981) and used as 1- to 3-day-old J2 for the bioassays.

The attachment and infection of M. javanica by P. penetrans: Pasteuria penetrans isolate P-20 (Oostendorp et al., 1990) used in this study was collected originally from females of *M. arenaria* parasitizing peanut in Levy County, Florida, and reared on *M. arenaria* growing on tomato in a greenhouse. Single-egg mass cultures were prepared for *M. arenaria* and *M. javanica* collected from peanut in the Levy County site. One- to three-day-old juveniles of each species were collected from the single egg mass cultures and each exposed separately to P-20 endospores. Five-hundred J2 of each species were placed in a 0.6-ml microfuge tube containing 50,000 endospores in 0.5 ml of water. The preparations were centrifuged for 5 minutes at 2,000 g (Micromax, International Equipment Company, Needham Hts, MA). Before the endospores were added they were exposed to sonication (FS14, Fisher Scientific, Suwanee, GA) for 5 minutes.

The first 20 spore-encumbered J2 of each species were arbitrarily selected, and the average number of endospores attached per J2 and percentage J2 with endospores attached were estimated by viewing with an inverted compound microscope at 400× magnification. To determine infection and development of the P-20 isolate on each root-knot nematode species, the spore-encumbered J2 (300 J2/cup) were inoculated on 4-week-old okra (*Hibiscus esculentus* l. cv. Clemson Spineless) seedlings growing in 8-cm-diam. insulated styrofoam cups. They were placed in a growth chamber and arranged in a randomized complete block with 10 replicates. An untreated control (no endospores attached) was included for comparison. The plants were kept at 29 °C, 60% humidity, and 12 hours of light provided by metal halide lamps. About 40 ml water was added daily alternating with water containing Peters Professional 20-20-20 (N, P_2O_5 and K_2O, respectively) (Scotts-Sierra Horticultural Products, Maryville, OH) fertilizer added at 0.2 g/liter of water. After 45 days plants roots were removed, washed free of soil individually, and incubated for 2 days in an enzymatic maceration solution (Charnecki, 1997). The samples were washed vigorously over stacked-sieves with 600-µm-pore openings (top) and 75-µm-pore openings (bottom). Twenty females were hand-picked arbitrarily from the bottom sieve. These females were crushed on a glass slide, covered with a cover slip, and examined microscopically for the presence of *P. penetrans* endospores.

Statistical analysis: Data were subjected to analysis of variance (ANOVA) with SAS software (SAS Institute, Cary, NC), and treatment means were separated and compared using Duncan's multiple-range test. Linear regression analysis was by Microsoft Excel (Microsoft Corporation, Redmond, WA).

Results

Persistence and suppressiveness in field soil: Treatments affected the initial and final population densities of *Meloidogyne* spp. (*M. arenaria* and *M. javanica*) ($P \leq 0.05$) (Table 1). Also, population densities (Pi, Pm, and Pf) were different among years, and there was a treatment x year interaction ($P \leq 0.05$) (Table 1). In the first year, initial densities were greater in the weed fal-

TABLE 1. The initial (Pi), mid-season (Pm), and final (Pf) population densities of *Meloidogyne* spp.[a] per 100 cm^3 of soil taken from peanut grown over four consecutive summer seasons (1999–2002) following 9 years of bahiagrass, rhizomal peanut, and weed fallow treatments.

Treatments[b]	Pi	Pm	Pf
		1999	
Bahiagrass	1 b	31 b	183 a
Rhizomal peanut	4 b	40 b	2 b
Weed fallow	17 a	147 a	214 a
		2000	
Bahiagrass	13 ab	172 a	161 a
Rhizomal peanut	1 b	49 b	41 b
Weed fallow	25 a	165 a	106 a
		2001	
Bahiagrass	15 a	137 a	459 a
Rhizomal peanut	11 a	123 a	200 b
Weed fallow	13 a	98 a	309 ab
		2002	
Bahiagrass	44 a	109 ab	130 a
Rhizomal peanut	34 a	193 a	155 a
Weed fallow	35 a	51 b	177 a
		ANOVA	
Treatments (T)	*	ns	*
Years (Y)	*	*	*
T × Y	*	*	*

[a] *Meloidogyne arenaria* race 1 is the dominant species infecting peanut at this site; however, *M. javanica* also was discovered infecting peanut in this field in autumn 2001.

[b] Main treatments were established in 1991 and were left with minimum disturbance until 1999. In summer 1999, bahiagrass and weed fallow were plowed under for planting peanut; however, the rhizomal peanut could not be plowed under because of its heavy thatch layer. Glyphosate herbicide was sprayed over the rhizomal peanut and the plots were prepared for planting peanut in 2000. Bahiagrass and weed fallow plots were planted with peanut during summers 1999 to 2002, whereas rhizomal peanut plots were planted during summers 2000 to 2002.

Data are an average of 10 replications. Means within each column in the same year with the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

ns = nonsignificant at $P \leq 0.05$.

TABLE 2. The percentage of second-stage juveniles (J2) of *Meloidogyne* spp.[a] with endospores of *Pasteuria penetrans* attached and the average number of endospores attached per J2 in 100 cm^3 of soil collected from each peanut plot before planting and at harvest for four growing seasons (1999–2002) following 9 years of bahiagrass, rhizomal peanut, and weed fallow treatments.

Treatments[b]	Percentage of J2 with endospores attached	Average number of endospores/J2	Percentage of J2 with endospores attached	Average number of endospores/J2
	Spring 1999		Autumn 1999	
Bahigrass	0 a	0.0 a	0 a	0.0 a
Rhizomal peanut	0 a	0.0 a	0 a	0.0 a
Weed fallow	2 a	0.1 a	0 a	0.0 a
	Spring 2000		Autumn 2000	
Bahigrass	1 a	0.1 a	10 b	1.1 a
Rhizomal peanut	0 a	0.0 a	3 b	0.3 a
Weed fallow	7 a	0.2 a	32 a	0.1 a
	Spring 2001		Autumn 2001	
Bahigrass	3 a	0.1 a	7 b	0.2 b
Rhizomal peanut	2 a	0.1 a	2 b	0.3 b
Weed fallow	7 a	0.1 a	75 a	5.8 a
	Spring 2002		Autumn 2002	
Bahigrass	12 ab	0.3 a	29 a	2.1 ab
Rhizomal peanut	4 b	0.1 a	21 a	0.6 b
Weed fallow	19 a	0.3 a	39 a	4.2 a
		ANOVA		
Treatments (T)	*	*	*	*
Years (Y)	*	*	*	*
T × Y	*	*	*	*

[a] *Meloidogyne arenaria* race 1 is the dominant species infecting peanut at this site; however, *M. javanica* also was discovered infecting peanut in this field in autumn 2001.

[b] Main treatments were established in 1991 and were left with minimum disturbance until 1999. In summer 1999, bahiagrass and weed fallow were plowed under for planting peanut; however, the rhizomal peanut could not be plowed under because of its heavy thatch layer. Bahiagrass and weed fallow plots were planted with peanut during summer 1999 to 2002, whereas rhizomal peanut plots were planted during summers 2000 to 2002.

Data are an average of 10 replications with 20 J2 per replication. Means with the same letter within each column in the same year and the same season are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

ns = nonsignificant at $P \leq 0.05$.

low plots than in the bahiagrass and rhizomal peanut plots ($P \leq 0.05$). In 2000, the Pi density remained low in the rhizomal peanut compared with weed fallow plots ($P \leq 0.05$). In general, the densities of *Meloidogyne* spp. remained relatively low throughout the course of the experiment; however, there was an immediate increase in the abundance of this nematode in the bahiagrass plots compared to the weed fallow plots at harvest. *Meloidogyne* spp. reached their highest densities numerically at the Pf of the third year (2001) in all three treatments. Root-knot nematode densities increased in the rhizomal peanut during the third and fourth seasons.

Treatment, years, and the treatment by year interactions affected the percentage of J2 with endospores attached and the average number of endospores attached per J2 in both spring and autumn seasons ($P \leq 0.05$) (Table 2). The percentage of J2 with endospores attached and the average number of endospores per J2 were less than 2 and 0.1, respectively, in all plots in 1999 and in spring 2000; however, they increased in all plots in autumn 2001, with the largest increase occurring in the weed fallow plots ($P \leq 0.05$) (Table 2). In spring 2002, the percentage of J2 with endospores attached was lower in the rhizomal peanut plots ($P \leq 0.05$) than in the weed fallow plots, and also in autumn there were fewer endospores attached per J2 in the rhizomal peanut plots compared with the weed fallow plots ($P \leq 0.05$) (Table 2).

Soil bioassays conducted over the 4 years showed a positive linear relationship with *Pasteuria* endospores and sampling dates (Fig. 1). There was an increase in the number of endospores per J2 in all plots over the 4-year period (weed fallow: $Y = 0.4461x - 0.6698$, $r^2 = 0.8755$, $P \leq 0.05$; bahiagrass: $Y = 0.3068x - 0.7568$, $r^2 = 0.8096$, $P \leq 0.05$; and rhizomal peanut: $Y = 0.1452x - 0.3978$, $r^2 = 0.6179$, $P \leq 0.05$). The greatest increase in densities of endospores occurred in the weed fallow plots, and the lowest densities occurred in the rhizomal peanut plots ($P \leq 0.05$) (Fig. 1).

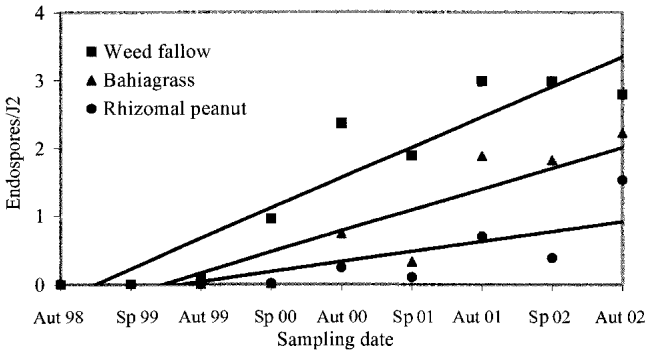

FIG. 1. Bioassay of the number of endospores of *Pasteuria penetrans* attached to second-stage juveniles (J2) of *Meloidogyne arenaria* race 1 in soil collected at planting and at harvest of peanut grown each year for 4 consecutive years in weed fallow and bahiagrass and for 3 years in rhizomal peanut plots. Data were transformed with $\log_{10}(x+1)$ before analysis. A linear model described a positive response between log-transformed *P. penetrans* endospore numbers and sampling dates for each of three treatments. Weed fallow: Y = 0.4461x – 0.6698, r^2 = 0.8755, $P \leq 0.05$; bahiagrass: Y = 0.3068x – 0.7568, r^2 = 0.8096, $P \leq 0.05$; and rhizomal peanut: Y = 0.1452x – 0.3978, r^2 = 0.6179, $P \leq 0.05$ (Aut = Autumn; Sp = spring).

Treatments affected the number of endospores per J2 in all the six-endospore categories except for category 1 (1 to 2 endospore/J2) ($P \leq 0.05$) (Table 3). Also, years affected the number of endospores per J2 in all endospore categories except for category 2 (3 to 5 endospore/J2) over the 4-years ($P \leq 0.05$) (Table 3). There was a treatment × year interaction among all five categories ($P \leq 0.05$) (Table 3). In 1999, weed fallow plots supported a greater percentage of J2 with endospores attached and average number of endospores per J2, with the greatest numbers observed in categories (0 to 3) ($P \leq 0.05$) (Table 3). In 2000, there were differences in percentage of J2 with endospores attached and endospores per J2 among all three treatments, with the greatest numbers occurring in weed fallow plots ($P \leq 0.05$) (Table 3). The greatest percentage of J2 with endospores attached was observed in weed fallow plots in 2000 (87%). The largest average number of endospores per J2 was observed each season in weed fallow plots; however, in the final season there was no difference between bahiagrass and weed fallow. The third and fourth years supported the greatest percentage of J2, with endospores attached in all treatments and the greatest numbers of endospores attached per J2.

There was an effect of treatment, years, and treatment x year interaction on galling percentages in 1999 ($P \leq 0.05$) (Table 4). The gall ratings on peanut (>50%) were consistently high for each treatment over the 4 years; but in 2002 the least amount of galling occurred in the weed fallow plots ($P \leq 0.05$) (Table 4). Galling was high on peanut grown in bahiagrass plots even the first year and remained fairly consistent over the 4 years, whereas in the rhizomal peanut plots galling was around 50% in years 2 and 3 but increased to over 90% in the fourth year (Table 4).

No difference occurred among treatments on the plant growth ratings within a year, but year as well as treatment × year interaction affected plant growth rating ($P \leq 0.05$) (Table 4). The growth rating of peanut was low in all plots throughout the study except for the rhizomal peanut plots in 2000 and 2001 ($P \leq 0.05$) (Table 4). Differences in growth ratings among the treatments were detected in each of the 4 years except for 1999 ($P \leq 0.05$) (Table 4).

The yield attained each crop year was low, which reflected that root knot disease was damaging. Although yields varied from year to year, there were differences among treatments ($P \leq 0.05$) (Table 4). There also was a difference among years, and treatment × year interaction on yield ($P \leq 0.05$) (Table 4). In 1999, peanut yield was higher in bahiagrass plots than in weed fallow plots ($P \leq 0.05$), and this trend continued in 2000. However, in 2001 yield was less in bahiagrass than in weed fallow plots ($P \leq 0.05$) (Table 4). The highest increase in peanut yield among treatments was observed in rhizomal peanut plots in 2001, the last year of recorded yield data ($P \leq 0.05$) (Table 4).

Treatment, years, and treatment × year interaction affected the percentage of *P. penetrans*-infected females for the last 3 years of the 4-year study ($P \leq 0.05$) (Table 5). In 1999, no endospore-infected females were recorded, but for the last 3 years, the percentage of endospore-infected females consistently increased in numbers in all plots over the course of the study (Table 5). In 2000, weed fallow plots supported a greater number of endospore-infected females then bahiagrass or rhizomal peanut plots, and this trend held for the fallowing 2 years ($P \leq 0.05$) (Table 5). Rhizomal peanut plots maintained the lowest number of endospore-infected females in both 2001 and 2002 ($P \leq 0.05$) (Table 5). Bahiagrass plots supported the greatest increase in the number of endospore-infected females, increasing from 6% in 2000 to 41% in 2002 (Table 5).

Suppressive soil test: In the soil from weed fallow and rhizomal peanut plots, root galling was less in the air-dried and untreated treatments than in the autoclaved and microwaved treatments ($P \leq 0.05$) (Table 6), whereas in the soil from bahiagrass plots, root galling was less only in the air-dried treatment ($P \leq 0.05$).

Regardless of the soil source (bahiagrass, rhizomal peanut, and weed fallow plots), peanut grown in the autoclaved soil treatments contained more eggs than any other treatments ($P \leq 0.05$) (Table 6). The number of eggs was not different in the microwaved and untreated soil for any treatment. For the air-dried soil, there were no eggs from the weed fallow and bahiagrass sites, but the number of eggs from the rhizomal peanut site was not different from the numbers from microwaved or untreated soil.

The main effects of soil source, treatment, and inoculation densities varied in the study. Soil sources did not affect the number of galls and eggs when both inocu-

TABLE 3. Soil collected at peanut harvest (1999–2002) was used to conduct a bioassay[a] of the percentage of second-stage juveniles (J2) of *Meloidogyne arenaria* race 1 with endospores of *Pasteuria penetrans* attached and the average number of endospores attached per J2 following 9 years of bahiagrass, rhizomal peanut, and weed fallow treatments.

Treatments[b]	Percentage of J2 with endospores attached[c]	Endospores/J2[c]	Category[d] (endospores/J2)					
			0 (None)	1 (1–2)	2 (3–5)	3 (6–15)	4 (16–100)	5 (>100)
			1999					
Bahiagrass	2 b	0.2 b	98 a	2 b	0 b	0 b	0 a	0 a
Rhizomal peanut	2 b	0.2 b	98 a	2 b	0 b	0 b	0 a	0 a
Weed fallow	63 a	0.9 a	27 b	38 a	22 a	13 a	0 a	0 a
			2000					
Bahiagrass	50 b	0.8 b	50 b	30 a	13 b	6 c	1 b	0 a
Rhizomal peanut	22 c	0.3 c	80 a	19 b	1 c	0 b	0 b	0 a
Weed fallow	87 a	2.4 a	23 c	25 ab	20 a	17 a	15 a	0 a
			2001					
Bahiagrass	63 ab	1.9 b	39 ab	10 b	10 a	20 a	21 b	0 a
Rhizomal peanut	47 b	0.7 c	53 a	24 a	12 a	6 b	5 c	0 a
Weed fallow	79 a	3.0 a	27 b	3 b	11 a	13 ab	45 a	1 a
			2002					
Bahiagrass	63 ab	2.2 a	36 ab	5 a	11 a	16 a	31 ab	1 a
Rhizomal peanut	53 b	1.5 b	46 a	12 a	12 a	16 a	14 b	0 a
Weed fallow	77 a	2.8 a	21 b	8 a	11 a	13 a	45 a	2 a
			ANOVA					
Treatments (T)	ns	*	*	ns	*	*	*	*
Years (Y)	*	*	*	*	ns	*	*	*
T × Y	*	*	*	*	*	*	*	*

[a] Forty grams of air-dried soil was placed in a 50-ml sterile polyethylene centrifuge tube. Five hundred 1- to 3-day-old *Meloidogyne arenaria* race 1 J2 were added. Three days later, the J2 were extracted by a centrifugal-flotation method, and the number of endospores attached per J2 was counted based on the first 20 J2 observed per sample.

[b] Main treatments were established in 1991 and were left with minimum disturbance until 1999. In summer 1999, bahiagrass and weed fallow were plowed under for planting peanut; however, the rhizomal peanut could not be plowed under because of its heavy thatch layer. Glyphosate herbicide was sprayed over the rhizomal peanut and the plots were prepared for planting peanut in 2000. Bahiagrass and weed fallow plots were planted with peanut during summers 1999 to 2002, whereas rhizomal peanut plots were planted during summers 2000 to 2002.

[c] Data are means of four replications with 20 J2 per replication, and they were transformed with $\log_{10}(x+1)$ before analysis. Means within a column in the same year followed by the same letter are not different according to Duncan's multiple-range test ($P \leq 0.05$).

[d] Percentage of J2 with endospore attached based on 20 nematodes per plot divided by six categories (category 0 = no endospores attached per J2, category 1 = 1 to 2 endospores attached per J2, 2 = 3 to 5, 3 = 6 to 15, 4 = 16 to 100, and category 5 = >100 endospores attached per J2). Data were transformed with arcsin ($\sqrt{x}$) before analysis, but untransformed arithmetic means are presented.

ns = nonsignificant at $P \leq 0.05$.

TABLE 4. Comparison of overall root-knot nematode galling percentages, plant growth ratings, and yields of peanut grown each year from 1999 to 2002 following 9 years of bahiagrass, rhizomal peanut, and weed fallow.

Treatments[a]	1999			2000			2001			2002		
	Galling (%)[b]	Growth rating[c]	Yield (kg/plot)	Galling (%)	Growth rating	Yield (kg/plot)	Galling (%)	Growth rating	Yield (kg/plot)	Galling (%)	Growth rating	Yield (kg/plot)
Bahiagrass	87 a	3.8 a	19 a	96 a	3.6 a	5.0 b	80 a	3.2 c	13 c	72 b	3.2 ab	ND
Rhizomal peanut	nd	nd	nd	55 b	8.0 a	12 a	53 b	6.7 a	22 a	94 a	2.3 b	ND
Weed fallow	85 a	3.5 a	12 b	99 a	3.3 b	3.0 c	71 a	4.9 b	16 b	52 c	3.8 a	ND
					ANOVA							
Treatments (T)	*	ns	ns									
Years (Y)	*	*	*									
T × Y	*	*	*									

[a] Main treatments were established in 1991 and were left with minimum disturbance until 1999. In summer 1999, bahiagrass and weed fallow were plowed under for planting peanut; however, the rhizomal peanut could not be plowed under because of its heavy thatch layer. Bahiagrass and weed fallow plots were planted with peanut during summers 1999 to 2002, whereas rhizomal peanut plots were planted during summers 2000 to 2002.

[b] Root-knot nematode galling percentages were determined on 26 plants per plot at harvest based on a scale of 0 to 100 (0 = no galls; 10 = 10% of root system galled, . . . 100 = 100% of root system galled).

[c] Peanut growth was rated on a scale of 1 to 10 (1 = stunted, chlorotic, or dead plants; 10 = full, green, lush growth).

ND = No data because of drought conditions in 2002.

nd = No data because of heavy thatch layer prevented plowing under the rhizomal peanut plots. Glyphosate herbicide was sprayed over the rhizomal peanut and the plots were prepared for planting peanut in 2000.

Data are an average of 10 replications. Means within each column with the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

ns = nonsignificant at $P \leq 0.05$.

TABLE 5. Percentage of *Pasteuria penetrans* endospore-filled females[a] of *Meloidogyne arenaria* race 1 extracted from peanut roots grown during the 2000–2002 seasons following 9 years of bahiagrass, rhizomal peanut, and weed fallow.

	Percentage of endospore-filled females		
Treatments[b]	2000	2001	2002
Bahigrass	6.0 b	11.8 b	41.2 b
Rhizomal peanut	0.3 b	1.3 c	16.0 c
Weed fallow	42.5 a	53.3 a	56.0 a
		ANOVA	
Treatments (T)	*	*	*
Years (Y)	*	*	*
T × Y	*	*	*

[a] The first 40 females were observed for the presence of endospores inside their body. No data were reported for 1999 because no females were found with endospores.

[b] Main treatments were established in 1991 and were left with minimum disturbances until 1999. In summer 1999, bahiagrass and weed fallow were plowed under for planting peanut; however, the rhizomal peanut could not be plowed under because of its heavy thatch layer. Glyphosate herbicide was sprayed over the rhizomal peanut and the plots were prepared for planting peanut in 2000. Bahiagrass and weed fallow plots were planted with peanut during summers 1999 to 2002, whereas rhizomal peanut plots were planted during summers 2000 to 2002.

Data are an average of 10 replications. Means within each column with the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

ns = nonsignificant at $P \leq 0.05$.

TABLE 6. Gall percentages, and number of eggs on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) grown in *Pasteuria penetrans*-infested soil that was treated by autoclaving, microwaving, air-drying, and untreated after inoculation with 0 or 2,500 second-stage juveniles of *Meloidogyne arenaria* race 1. The soil was collected from peanut plots following 9 years of growing bahiagrass, rhizomal peanut, and weed fallow and then 4 years of continuous peanut.

		Galling (%)[c] Inoculation density		Number of eggs[d] Inoculation density	
Soil source[a]	Soil treatment[b]	0	2,500	0	2,500
Weed fallow	Autoclaved	0 b	60 a	0 a	1,150 a
	Microwaved	0 b	55 a	0 a	180 b
	Air-dried	0 b	17 b	0 a	0 c
	Untreated	4 a	20 b	0 a	90 b
Bahiagrass	Autoclaved	2 a	50 a	0 a	11,910 a
	Microwaved	5 a	45 a	0 a	260 b
	Air-dried	0 b	15 b	0 a	0 c
	Untreated	0 b	40 a	0 a	120 b
Rhizomal peanut	Autoclaved	0 b	60 a	0 a	6,640 a
	Microwaved	0 b	40 a	0 a	110 b
	Air-dried	0 b	10 b	0 a	10 b
	Untreated	0 b	15 b	0 a	60 b

[a] Soils were taken from three treatments (bahiagrass, rhizomal peanut, and weed fallow), which were established in 1991 and left with minimum disturbance until 1999. In summer 1999, bahiagrass and weed fallow were plowed under for planting peanut; however, the rhizomal peanut could not be plowed under because of its heavy thatch layer. Glyphosate herbicide was sprayed over the rhizomal peanut and the plots were prepared for planting peanut in 2000. Bahiagrass and weed fallow plots were planted with peanut during summers 1999 to 2002, whereas rhizomal peanut plots were planted with peanut during summers 1999 to 2002, whereas rhizomal peanut plots were planted during summers 2000 to 2002.

[b] Autoclaved = treated twice at 55 kPa for 99 minutes each. Microwaved = treated at full power for 3 minutes/kg of soil. Air-dried = left 2 weeks on a greenhouse bench. Untreated samples stored in a cool room at 10 °C until used.

[c] Root galling was determined at harvest based on a scale of 0 to 100 (0 = no galls, 10 = 10% of root system galled, . . . 100 = 100% of root system galled).

[d] Eggs were extracted from galled roots by dissolving the gelatinous matrices with 0.5% sodium hypochlorite and catching individual eggs on a sieve with 25-µm pore openings.

Data are means of five replications. Means within a column in the same soil source followed by the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

lum levels were included in the analysis (Table 7). The soil treatments and inoculation levels together affected the number of galls and eggs if the inoculum level of 0 nematode is excluded because it produces only negative data. The effect of soil sources differed in the number of eggs but not with the number of galls when only the inoculum level of 2,500 J2 was considered ($P \leq 0.01$). There was a combined effect of soil sources and treatment on the number of galls and number of eggs ($P \leq 0.01$) (Table 7). However, no difference was observed in the untreated soil.

The attachment and infection of M. javanica by P. penetrans: Percentage of J2 with endospores attached and the average number of endospores per J2 of *M. javanica* were low as compared to those of *M. arenaria* ($P \leq 0.01$) (Table 8). There was no infection of *M. javanica* by *P. penetrans;* however, the infection rate of *M. arenaria* by *P. penetrans* was 30%.

Discussion

Persistence and suppressiveness in field soil: Peanut grown continuous over 4 years in bahiagrass, rhizomal peanut, and weed fallow plots affected the number of J2 of *M. arenaria* and endospore densities of *P. penetrans*. When peanut was planted in the beginning of our evaluations, *P. penetrans* was detected in low numbers in the soil but the number of root-knot nematodes also was low. Densities of endospores are likely dependent on root-knot nematode densities (Dickson et al., 1991, 1994; Sayre, 1993). Our bioassays indicated that the presence of *P. penetrans* in the soil could be detected with greater precision than by detecting endospore-encumbered J2 extracted from soil. Air-drying of soil, the first step of the bioassay procedure, could enable spores to attach more readily. Also, air-drying may inhibit or terminate other microbial activities that would hinder endospore attachment, or it may be a matter of maturation of endospores, as it is known that mature endospores that have shed their exosporium coat readily attach to their nematode host (Davies et al., 1988; Stirling, 1985). Also, bringing the soil moisture content up to 100% in a bioassay positively influences attachment (Brown and Smart, 1984).

Over the course of the experiment, weed fallow plots supported the greatest endospore densities, whereas rhizomal peanut resulted in the lowest densities, with bahiagrass being intermediate. Since neither bahiagrass nor rhizomal peanut is a host for *M. arenaria,* they are suggested as rotation crops for management of that nematode in peanut (Norden et al., 1977; Summer et al., 1999). In weed fallow plots hairy indigo and alyce

TABLE 7. Analysis of variance table for the effect of soil sources (soil taken from bahiagrass, rhizomal peanut, and weed fallow plots), and soil treatments (autoclaved, microwaved, air-dried, and untreated), based on *Meloidogyne arenaria* race 1 inoculum densities of 0 and 2,500 second-stage juveniles (J2) or 2,500 J2 alone on number of galls and nematode reproduction following 9 years of growing bahiagrass, rhizomal peanut, weed fallow and then 4 years of continuous peanut.

Source of variation	Number of galls	Number of eggs
Two inoculum levels: 0 and 2,500 J2		
Soil source (S)	ns	ns
Soil treatment (T)	**	**
Inoculation density (I)	**	**
S × T	*	**
S × I	**	**
T × I	**	**
S × T × I	**	**
One inoculum level: 2,500 J2		
Soil source (S)	ns	**
Soil treatment (T)	**	**
S × T	**	**

ns = nonsignificant.

clover were observed supporting root-knot nematode infection. The hairy indigo population was dense in the weed fallow plots and a few plants occurred in the bahiagrass plots, but there were none observed in the rhizomal peanut plots. While hairy indigo has been reported to suppress *M. javanica* and *M. incognita* (McSorley et al., 1994; Rodríguez-Kábana et al., 1988), this weed is susceptible to *M. arenaria* race 1. A few plants of alyce clover also were observed to be heavily galled in weed fallow plots. This plant is known to support development of the commonly occurring root-knot nematodes. Development of *M. arenaria* on these two weeds would most likely sustain *P. penetrans*. The increases in endospore densities became more apparent among all treatments as the study progressed over 4 years despite the fact that the endospore densities were almost undetectable at the beginning in 1999. It should be pointed out, however, that with the current methods available for detecting *P. penetrans* in soil, only a low precision of detection is thought to be possible. Nonetheless, over the course of 4 years of continuous peanut there was no strong evidence that endospore densities reached suppressive levels because peanut roots, pods, and pegs were generally heavily galled in all plots, and yields appeared to be suppressed. Unfortunately, in the final year of the experiment an extended drought caused extreme stress on plants to the point where harvesting was not possible. Additionally, the examination of numerous peanut roots at harvest revealed infection by *Rhizoctonia solani* Kuhn. This fungal agent occurs worldwide and causes stand losses when abundant (Porter et al., 1984). The density of this organism on peanut was not determined, but it was not surprising that the disease incidence was high because the previous history of peanut culture in the field was mostly a monoculture from 1969 through 1990, and then during 1999 to 2002. Nonetheless, over the course of the study, peanut yields were better in the bahiagrass and rhizomal peanut plots than in the weed fallow plots.

TABLE 8. The percentage of second-stage juveniles (J2) of *Meloidogyne javanica*[a] and *Meloidogyne arenaria*[a] with endospores of *Pasteuria penetrans* attached, average endospores attached per J2, and percentage of endospores-filled females from galled roots of okra, *Hibiscus esculentus* L. cv. Clemson Spineless.

Treatments	Percentage of J2 with endospores attached[b]	Endospores/J2[b]	Percentage of endospore-filled females[c]
M. arenaria	59 a	2.8 a	30 a
M. javanica	31 b	0.8 b	0 b

[a] Both nematode species originated from peanut grown in experimental research farm, near Williston, Florida. A single egg mass culture of each was used in this experiment.

[b] The first 20 J2 were observed for the presence of endospores attached to their body.

[c] The first 20 females were observed for the presence of endospores inside their body.

Data are means of 10 replications. Means within a column followed by the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

Two successful examples of the biological control potential of *P. penetrans* for management of peanut root-knot nematode on peanut were reported when the bacterial agent was introduced to field microplots (Chen et al., 1996; Oostendorp et al., 1990; 1991). These two studies demonstrated that *P. penetrans* suppressed the peanut root-knot nematode in a period of 2 or 3 years and resulted in the soil having low numbers of J2, low root, pod and peg galling, and increased peanut yield. In another example, *P. penetrans* suppressed the peanut root-knot nematode beginning from the third season of a 7-year study (Weibelzahl-Fulton, 1998).

The long-term persistence of *P. penetrans* and suppressiveness to root-knot nematodes in soil is an unknown. This was the first attempt to study the effects of different crops on the development of *P. penetrans* and nematode populations in natural field soil, i.e., without the introduction of either root-knot nematode or *P. penetrans*. Over the 4-year study, where peanut was grown continuous each year there were relatively low root-knot nematode population densities, which may be attributed to infection and suppression by *P. penetrans*. But the discovery of *M. javanica* infecting peanut at this site following harvest in 2001 was a complicating factor in interpreting our results (Cetintas et al., 2003). Its detection means that a portion of peanut yield loss each year could be attributed to this nematode, and its presence could mean that soil suppressiveness by *P. penetrans* as previously reported (Dickson et al., 1991) could not be achieved. Our study showed that the P-20 isolate of *P. penetrans* did not develop in *M. javanica*. Earlier studies showed that P-20 did not develop in *M. javanica,* but in this case the isolate of *M. javanica* was not a parasite of peanut (Oostendorp et al., 1990).

Suppressive soil test: More studies have been conducted on fungal antagonists of nematodes causing soil sup-

pressiveness than bacterial agents of nematodes (Kerry, 1982; Stirling and Mankau, 1979; Westphal and Becker, 2000). Of the many nematode antagonists, *P. penetrans* has attracted a great deal of interest over the past 2 decades, especially as it relates to root-knot disease of peanut (Chen et al., 1997; Dickson, 1998; Dickson et al., 1991, 1994; Freitas et al., 2000). Fecundity of root-knot nematodes may be reduced up to 94% in soils where *P. penetrans* has been applied (Gowen et al., 1989). Dickson et al. (1991) reported that a root-knot nematode-infested site that had a damaging nematode population density eventually became highly suppressive by *P. penetrans.* A suppressive soil test revealed a suppressive agent that was believed to be *P. penetrans* in the air-dried or untreated soil (Dickson et al., 1994). However, in most of the earlier work formalin was used as a method to reduce possible fungal antagonists. In this study, microwaving was used as an improved method for reducing fungal antagonists. Studies report that microwaving soil is an effective method to reduce possible fungal antagonists (Chen et al., 1995; Ferris, 1984; Weibelzahl-Fulton et al., 1996). The time required for effective treatment with microwaving varies with soil moisture and output of the microwave oven (Ferris, 1984). In this study the number of galls was high in the microwave treatment, but the number of eggs was relatively low. Thus, fecundity was apparently reduced because large numbers of females were filled with endospores. The fact that microwave treatment had a higher incidence of galling than occurred in air-dried or untreated soil indicates endospore attachment capability or their infectivity may have been affected (Weibelzahl-Fulton et al., 1996). In air-dried and untreated soils, except for the untreated soil from bahiagrass, there was a reduction in galling. When *P. penetrans* is at high densities and more than 1 to 5 endospores attach per J2, mobility is greatly reduced (Davies et al., 1991). For each treatment of soil from bahiagrass, rhizomal peanut, and weed fallow there remained a suppressive level of *P. penetrans;* however, soil from weed fallow retained a higher density of *P. penetrans* than either bahiagrass or rhizomal peanut. This suggests that for *P. penetrans* to remain at maximum suppressive levels, some amplification of the bacterium must occur as might be provided by root-knot nematode infection on weed hosts. Gowen et al., (1989) reported that *P. penetrans*-infected females are able to form giant cells and galls, but those females are unable to produce eggs because their fecundity is reduced by *P. penetrans* infection. Microwaving soil possibly aided in separating suppressiveness caused by fungal agents and *P. penetrans,* but we cannot be sure because we did not attempt to isolate possible fungal agents.

We assume that autoclaving killed all biological agents as previously reported (Chen et al., 1995; Weibelzahl-Fulton et al., 1996), thereby allowing root-knot nematodes to be infective; whereas air-drying and untreated soils contained antagonists that were suppressive to *M. arenaria.* Data from the bioassays (the field study) showed that *P. penetrans* was again found to be at a suppressive level in the soil based on the suppressive test, but from field results there was no conclusive evidence that the same degree of suppressiveness as that reported earlier still occurred even through relatively high numbers of endospores were present at the conclusion of the field study (Dickson et al., 1994).

It was not possible to separate the galling induced by the two root-knot nematode species infesting this field site. The level of infection on peanut in 2002 by *M. javanica* is not known, but the frequency of *M. javanica* among the root-knot nematode females extracted from peanut roots taken at harvest ranged from 5% to 29% (Cetintas et al., 2003). The level of suppressiveness in the suppressive soil test was clearly higher than the level we observed in the field (the field study), but this may be attributed to *M. javanica.* These results suggest that *P. penetrans* may still be a major factor in suppressing the population density of *M. arenaria* even though the peanut yields and the amount of galling on the roots do not reflect a suppressive of this nematode. These results warrant additional long-term experiments with extended time to aid in the understanding of the dynamics of *P. penetrans* in field soil and to determine whether nematode population densities can be maintained at acceptable low levels.

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