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Suppression of *Meloidogyne arenaria* race 1 by Soil Application of Endospores of *Pasteuria penetrans*[1]

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Abstract: The potential of *Pasteuria penetrans* for suppressing *Meloidogyne arenaria* race 1 on peanut (*Arachis hypogaea*) was tested over a 2-year period in a field microplot experiment. Endospores of *P. penetrans* were mass-produced on *M. arenaria* race 1 infecting tomato plants. Endospores were inoculated in the first year only at rates of 0, 1,000, 3,000, 10,000, and 100,000 endospores/g of soil, respectively, into the top 20 cm of microplots that were previously infested with *M. arenaria* race 1. One peanut seedling was planted in each microplot. In the first year, root gall indices and pod galls per microplot were significantly reduced by 60% and 95% for 100,000 endospores/g of soil, and 20% and 65% for 10,000 endospores/g of soil, respectively. Final densities of second-stage juveniles (J2) in soil were not significantly different among the treatments. The number of endospores attached to J2 and percentage of J2 with attached endospores significantly increased with increasing endospore inoculation levels. *Pasteuria penetrans* significantly reduced the densities of J2 that overwintered. In the second year, root and pod gall indices, respectively, were significantly reduced by 81% and 90% for 100,000 endospores/g of soil, and by 61% and 82% of 10,000 endospores/g of soil. Pod yields were significantly increased by 94% for 100,000 and by 57% for 10,000 endospores/g of soil, respectively. The effect of *P. penetrans* on final densities of J2 in soil was not significant. Regression analyses verified the role of *P. penetrans* in the suppression of *M. arenaria*. The minimum number of endospores required for significantly suppressing *M. arenaria* race 1 on peanut was 10,000 endospores/g of soil.

Key words: Arachis hypogaea, bacterium, biological control, endospore, *Meloidogyne arenaria, Pasteuria penetrans,* peanut, root-knot nematode.

Peanut (*Arachis hypogaea* L.), one of the most important crops grown in the southern United States, is host to a virulent pathogenic nematode, *Meloidogyne arenaria* (Neal) Chitwood race 1 (23). In Texas, initial population densities of 8.8 to 16.6 second-stage juveniles (J2) of *M. arenaria* per 100 cm^3 soil were sufficient to cause 10% suppression of yield (31), whereas the damage threshold in Florida was determined to be as low as 1.0 J2/100 cm^3 soil (21). The management of *M. arenaria* relies largely on a combination of methods that includes nematicides and crop rotation (11,23). Economic considerations and potential environmental hazards of nematicides, however, have created a climate of uncertainty regarding their continued use on peanut. It is also documented that nematicides are often unreliable when nematode population densities are high (11), and some successful rotations must remain in place for more than 4 years (12).

Biological control offers an alternative or supplemental management tactic to chemical or cultural control of nematode pathogens on peanut. It is promising because bacterial parasites and numerous fungal parasites have been observed infecting nematodes in peanut fields in the southern United States (13). One nematode antagonist in particular, *Pasteuria penetrans* (Thorne) Sayre & Starr, has potential for biological control of root-knot nematodes (13,14). *Pasteuria penetrans* is an obligate, mycelial, endospore-forming bacterial parasite of *Meloidogyne* spp. Endospores of *P. penetrans* attach to the cuticle of the J2 of *Meloidogyne* spp. in soil. Infection occurs after the J2 enters a plant root, where parasitized nematodes are able to develop into females but are incapable of reproduction (2). The females and sometimes males (13) become filled with endospores, which are eventually released into the soil upon host disintegration.

Pasteuria penetrans has been observed in

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nematode-suppressive soils (13) and successfully tested in greenhouse (6, 10, 29) and field microplot experiments (4,13–15,24). Attempts to culture *P. penetrans* on artificial media have failed (3). The parasite must be reared on *Meloidogyne* spp., which, in turn, must be reared on susceptible plants or in a nematode-excised root system (30). Neither procedure provides production of sufficient inoculum for large-scale applications. Thus, use of this parasite for practical, successful control of the root-knot nematode under normal field cropping conditions has not been accomplished. Also, little information is available on the capability of *P. penetrans* to suppress *M. arenaria* on peanut. Our objective was to determine the efficacy of *P. penetrans* on the peanut root-knot nematode at varying soil endospore densities under field conditions.

Materials and Methods

Pasteuria penetrans *isolate:* An isolate of *P. penetrans* designated P-20 (24), originating from *M. arenaria* race 1 from a peanut field in Levy County, Florida, was propagated on *M. arenaria* growing on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) in a greenhouse. Juveniles (J2 up to 5 days old) with endospores attached were obtained by a centrifugation attachment method (17). Tomato plants (45 to 60 days old), which were grown in 15-cm-diameter pots, were inoculated three times with the endospore-attached J2 (1,000–1,500 J2/pot/time) at intervals of 1 week. Averaged over all inoculations, 96 ± 5% of the J2 had at least one endospore attached, with an average (±SD) of 4.9 ± 2.0 endospores/J2 for those J2 with endospores. These estimates were made by counting 20 J2/aliquot/each inoculation. Tomato plants were fertilized weekly with 1 g of 20-20-20 (N-P-K)/pot. Insecticide applications and water were provided as needed. At 45 to 60 days after the last inoculation, the root systems were harvested, rinsed, air-dried, and ground in a Wiley mill (Intermediate Model, Arthur H. Thomas, Philadelphia, PA) into a powder that passed through a sieve with 250-μm-pore openings. The root powder weighed approximately 900 g and was stored in a cool room at 13 °C. The endospore concentration were estimated at 79 million endospores/g of root powder by a machine disruption method followed by counting on a hemocytometer (7).

Nematode isolate: An isolate of *M. arenaria* from Levy County, Florida, was cultured on tomato in a steam-pasteurized potting soil. Eggs of *M. arenaria* were extracted (18) from roots in 0.5% sodium hypochlorite for 30 seconds and caught on a sieve with 25-μm-pore openings, rinsed, and placed on Baermann funnels. The roots and plant debris were caught and separated from eggs by a sieve with 75-μm-pore openings nested on a sieve with 25-μm-pore openings. Second-stage juveniles that hatched on the first day were discarded to avoid using nematodes that may have acquired endospores in the potting soil. The juveniles were collected by decanting the water suspension from Baermann funnels on a sieve with 25-μm-pore openings, rinsed, and transferred into a beaker. The number of J2 in the suspension was determined by counting five 1-ml aliquots with the use of a compound inverted light microscope.

Field testing: Pasteuria penetrans was evaluated as a biological nematicide over a 2-year period. The experiment had five endospore inoculation levels, 0, 1,000, 3,000, 10,000, and 100,000 endospores/g of soil, with 10 replicates each. Microplots of clay flue liners (Cement Precast Products, Gainesville, FL) were installed in rows of nine microplots with a distance of 0.9 m between microplots and rows in an Arredondo fine sand soil (94% sand, 1% silt, 5% clay; <0.1% organic matter; pH 5.6) (21) on the Green Acres Agronomy Farm, University of Florida, Gainesville. The soil profile consisted of a sand (≥90% sand) to a depth of >1.2 m. The flue liner dimension was 25 cm × 40 cm with a length of 100 cm, and each flue liner was

inserted 90 cm deep into the soil. The microplots were disinfected with 1,200 kg/ha of a mixture of 98% methyl bromide and 2% chloropicrin applied under 0.076-mm-thick polyethylene plastic mulch 3 months before adding inocula.

Meloidogyne arenaria race 1 was introduced into the microplots on 24 May 1994 and increased on tomato roots. Microplots were templated to make 15 uniformly spaced holes, five each with depths of 7, 14, and 21 cm. A suspension of 25,000 eggs of *M. arenaria* in 300 ml water was sprinkled evenly over the soil and incorporated 7- to 10-cm deep. The hatching rate of the eggs was estimated at 21.6%. One 50-day-old tomato plant (cv. Rutgers) was transplanted into each microplot and fertilized weekly with 0.9 g of 20-20-20 (N-P-K) beginning 7 days after inoculation. After 45 days, the aerial portion of each tomato plant was removed, and the roots chopped into 3- to 5-cm-long pieces before mixing back into the soil of the original microplot. After 2 weeks, soil samples were taken to determine the nematode population densities in each microplot.

On 22 July, the microplots were inoculated with *P. penetrans* endospores. Weighed root powder containing endospores was suspended in 300 ml of water and sprinkled onto the top 20 cm soil, which was removed from each microplot and mixed in a cement mixer (Stow Manufacturing Company, Oakland, CA) for 2 minutes before placing it back into the original microplot. On 12 August, soil samples were taken to determine the rate of endospores attached per J2. A 10-day-old peanut seedling (cv. Florunner) was transplanted and 1 g of *Rhizobium* spp. (The Nitragin Company, Milwaukee, WI) was dusted onto each microplot. Microplots were drip-irrigated. Insecticides and fungicides were applied as recommended, and each microplot was hand-weeded as needed. After 125 days, peanuts were harvested. Plant yield, root gall indices, and number of pod galls were recorded. Soil samples were taken to determine the final nematode population density. The root system was chopped into 2- to 5-cm-long pieces and immediately put back into the microplot from which it was removed. The microplots were planted with wheat (*Triticum aestivum* L. cv. Fla 304) as a winter cover crop.

Soil samples consisted of three cores (2.5-cm-diam. × 20-cm-deep) diagonally arranged in a microplot. Juveniles were extracted from 100 cm^3 soil using centrifugal flotation (19) and counted using a compound inverted light microscope. Root galling was indexed on a 0 to 10 scale based on the percentage of roots galled (0 = no galls on roots; 1 = 1 to 10% roots galled; 2 = 11 to 20% roots galled; . . ., 10 = 91 to 100% roots galled) (1). Pod galls were counted individually in 1994 and indexed on the 0 to 10 scale in 1995. Dry weights of foliage and pods were determined by drying at 60 °C for 1 week.

The experiment was continued in 1995. The wheat was cut and the roots mixed into the soil on 2 May. Two weeks later, soil samples were take and a 10-day-old peanut seedling (cv. Florunner) was transplanted into each microplot. Microplots were managed as in 1994, and data collection was similar. Plants were harvested on 19 September 1995.

Before data analysis, percentage and rating data were transformed by arcsin ($\sqrt{x}$). Nematode densities and numbers of endospores per J2 were transformed by $\log_{10}(x + 1)$. Dry weight of foliage + pods in 1994 was transformed by $\log_{10}x$ because the dataset had a binomial distribution. Data presented in the text were back-transformed. One microplot in the control was apparently contaminated by *P. penetrans* and thus excluded in statistical analysis. Although regression analysis is usually used with quantitative variables (22), our main objective was to make comparisons among the inoculation levels tested, and therefore Duncan's multiple-range test was used to compare differences among treatment means. Regression analysis was used, and an equation was pro-

vided if the correlation was significant at $P \leq 0.05$.

Results

Pasteuria penetrans reduced the root gall indices, pod gall index, and number of galls on pods and improved peanut production ($P \leq 0.05$) (Fig. 1, Table 1). The final J2 populations in soil were not affected by *P. penetrans* ($P > 0.05$) (Table 1).

1994 Experiment: Initial population densities among treatments before planting peanut were not different ($P > 0.05$) (Fig. 2A, Table 1). The low number of J2/100 cm^3 soil was due to a high mortality after the removal of host tomato, averaging 94%. Endospores readily attached to J2 of *M. arenaria* after 3 weeks of soil application. Number of endospores attached to J2 (Y_1) were different among the treatments ($P \leq 0.001$) (Fig. 2E, Table 1) and correlated with the different levels of endospore inoculations ($X = \log_{10}$ [inoculation level + 1]) ($\log_{10} Y_1 = -2.25 + 0.586X$, $r^2 = 0.867$, $P \leq 0.05$). Root gall index and pod galls per plot were reduced by soil application of endospores ($P \leq 0.05$) (Fig. 2C,D, Table 1). Compared to the control, root gall index and pod galls per plot were reduced to 40% and 5%, respectively, for 100,000 endospores/g of soil and 80% and 35%, respectively, for 10,000 endospores/g of soil. Low endospore levels of 1,000 and 3,000 endospores/g of soil had a slight increase in the root gall index and a decrease in pod galls per plot compared to the control. This inconsistency was probably due to experimental variation or the low number of endospores attached to J2 at planting (Fig. 2E). Dry weights of foliage + pods were increased 41 to 73% by all endospore inoculation levels compared to the control, but there was no difference among the treatments ($P > 0.05$) (Fig. 2G, Table 1). Final population densities of J2 in soil were not different among treatments ($P > 0.05$) (Fig. 2B, Table 1). Number of endospores attached to J2 (Y_2) and percentage of J2 with attached endospores at harvest (Y_3) were different ($P \leq 0.001$) and related to the endospore application levels ($\log_{10} Y_2 = -2.23 + 0.561X$, $r^2 =$

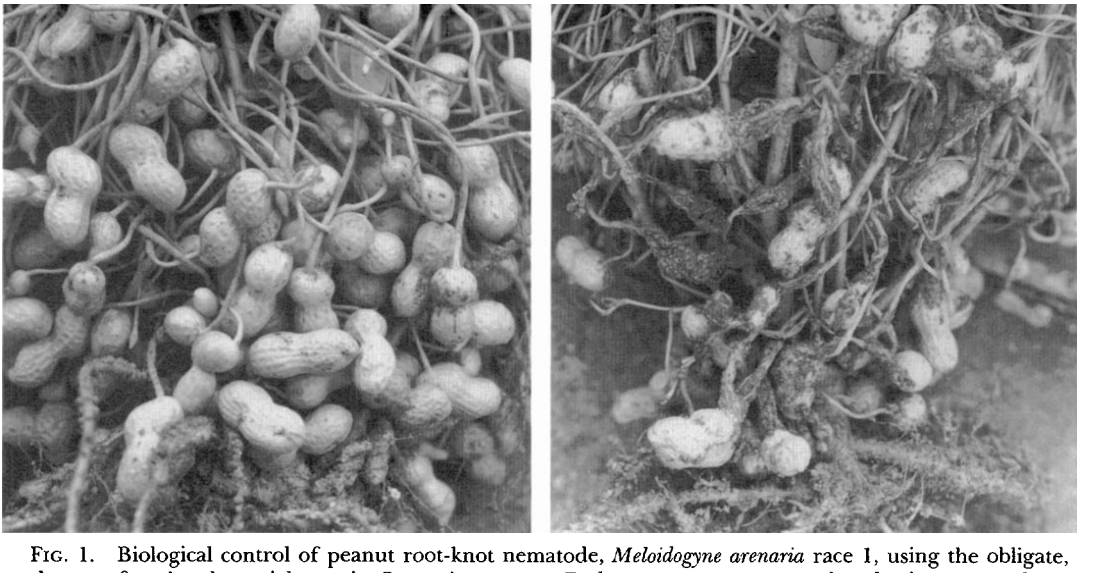

FIG. 1. Biological control of peanut root-knot nematode, *Meloidogyne arenaria* race 1, using the obligate, endospore-forming, bacterial parasite *Pasteuria penetrans*. Endospores were mass produced using a pot culture method, and inoculated in field microplots that were preinfested by *M. arenaria*. Photographs were taken in the second year following the inoculation. Right: The control. Peanut had few pods and the pods were severely galled by *M. arenaria*. Left: Microplot was inoculated with 100,000 endospores/g of soil. Peanut had few galls on pods.

TABLE 1. Summary of analysis of variance results for effects of *Pasteuria penetrans* on *Meloidogyne arenaria* race 1 and peanut yields.[a]

Data measured	1994	1995
J2/100 cm^3 soil at planting (Pi)	ns	$P \leq 0.01$
J2/100 cm^3 soil at harvest (Pf)	ns	ns
Root gall index	$P \leq 0.05$	$P \leq 0.001$
Pod gall index	—	$P \leq 0.001$
Pod galls/plot	$P \leq 0.001$	—
Dry weight of foliage + pods (g)	ns	—
Dry weight of foliage (g)	—	$P \leq 0.001$
Dry weight of pods (g)	—	$P \leq 0.01$
Endospores/J2 at planting	$P \leq 0.001$	—
Endospores/J2 at harvesting	$P \leq 0.001$	$P \leq 0.001$
% J2 with attached endospores at harvest	$P \leq 0.001$	$P \leq 0.001$

[a]Data show significance levels of analysis of variance; ns = not significant at $P \geq 0.05$; — = data not available. Percentage and rating scale were transformed by arcsin ($\sqrt{x}$). Population density of second-stage juveniles (J2) in soil and number of endospores per J2 data were transformed by $\log_{10}(x + 1)$. Dry weight of foliage + pods in 1994 had a binomial distribution and was transformed by $\log_{10} x$.

0.910, $P \leq 0.01$; $Y_3 = -6.92X + 4.23X^2$, $R^2 = 0.968$, $P \leq 0.01$) (Fig. 2F,H, Table 1). This indicated that *P. penetrans* persisted in the soil.

1995 Experiment: Pasteuria penetrans reduced the overwintered J2 population by planting time in 1995 ($P \leq 0.01$) (Fig. 3A, Table 1). The J2 in soil (Y_4) were correlated to the endospore inoculation levels ($Y_4 = 4.20 - 1.73X + 0.24X^2$, $R^2 = 0.991$, $P \leq 0.01$) (Fig. 3A). After planting peanut, the population densities of J2 in soil showed no differences among treatments ($P > 0.05$) (Fig. 3B, Table 1). *Pasteuria penetrans* reduced the root and pod gall indices ($P \leq 0.001$) (Fig. 3C,D, Table 1). Compared to the control, the root and pod gall indices were reduced to 19% and 10%, respectively, for 100,000 endospores/g of soil, and 39% and 18%, respectively, for 10,000 endospores/g of soil. Dry weights of pods was increased 94% and 58% at 100,000 and 10,000 endospores/g of soil, respectively, as compared to the control ($P \leq 0.01$) (Fig. 3G, Table 1). Dry weight of foliage was increased 75% in microplots with 100,000 endospores/g of soil ($P \leq 0.001$) (Fig. 3F, Table 1). *Pasteuria penetrans* again persisted in soil in 1995 (Fig. 3E,H).

Based on the regression analyses, there was a direct cause and effect of *P. penetrans* on the suppression of pod galls per plot in 1994 and pod gall index in 1995 (Fig. 4B,D). Regression analyses indicated that pod galls per plot (Y_5) and pod gall index (Y_6) were negatively correlated with the percentage of J2 attached with *P. penetrans* (Z) ($Y_5 = 46.4 - 0.39Z$, $r = 0.40$, $P \leq 0.001$; $Y_6 = 6.56 - 0.071Z$, $r = 0.57$, $P \leq 0.001$). However, final population densities of J2 in soil in 1994 and 1995 were not correlated with *P. penetrans* levels (Fig. 4A,C).

DISCUSSION

Pasteuria penetrans suppressed root knot on peanut in field microplots when applied before the cropping season. The increased yields and reduced galls resulting from adding *P. penetrans* to microplots containing *M. arenaria* demonstrated that *P. penetrans* reduced the damaging effects of *M. arenaria* on peanut. Many researchers have reported similar results with root-knot nematodes on other crops. Brown et al. (4) claimed a yield increase of 23–24% for tobacco and 38–55% for *Vicia villosa* in a 2-year experiment on *M. incognita*. Dube and Smart (15) reported a synergistic increase of plant yields in various crops when *Paecilomyces lilacinus* and *P. penetrans* were applied together to control *M. incog-*

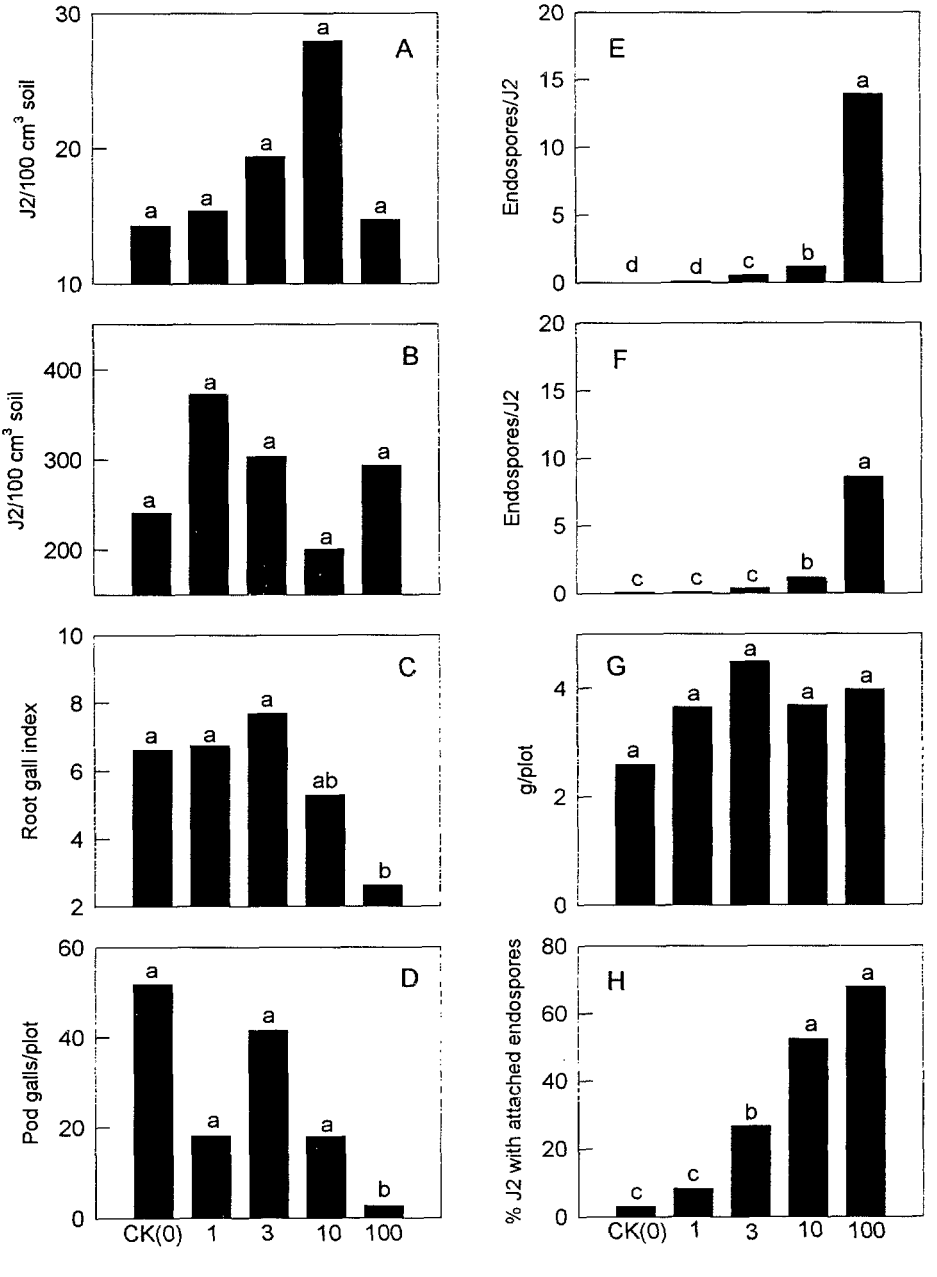

FIG. 2. Effect of *Pasteuria penetrans* inoculum levels on *Meloidogyne arenaria* population and peanut yield in 1994. Data are means of 10 replicates, except the control (nine replicates). Same letter on bars indicates no difference ($P > 0.05$) according to Duncan's multiple-range test. For all equations, $X = \log_{10}$ (endospore inoculum level + 1). A) Population density of second-stage juveniles (J2) in soil at planting. B) Soil J2 population at harvest. C) Root gall index (0 to 10 scale). D) numbers of galls on pods. E) Number of endospores attached to J2 at planting, 3 weeks after endospore inoculation. $\text{Log}_{10} Y = -2.25 + 0.586X$, ($r^2 = 0.867$; $P \leq 0.05$). F) number of endospores attached to J2 at harvest. $\text{Log}_{10} Y = 2.23 + 0.561X$, ($r^2 = 0.910$; $P \leq 0.01$). G) Dry weight of foliage + pods. H) Percentage of J2 with attached endospores at harvest. $Y = -6.92X + 4.23X^2$ ($R^2 = 0.968$; $P \leq 0.01$).

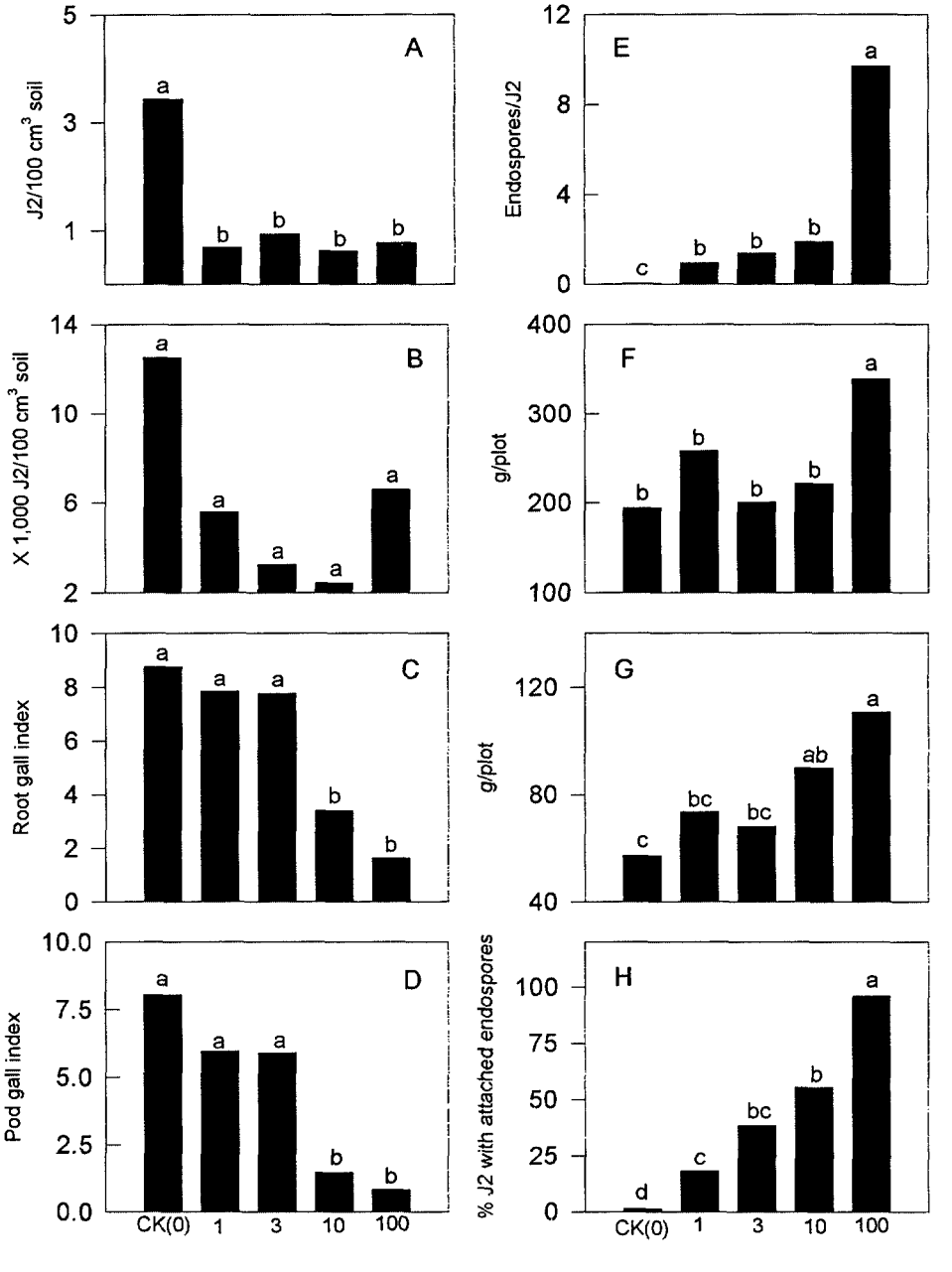

FIG. 3. Population changes of *Meloidogyne arenaria* race 1 and *Pasteuria penetrans* in 1995. This was the continued experiment following the endospore inoculation in 1994. Data are means of 10 replicates, except the control (nine replicates). Same letter on bars indicates no difference ($P > 0.05$) according to the Duncan's multiple-range test. For all equations, $X = \log_{10}$ (endospore inoculum level + 1). A) Population density of second-stage juveniles (J2) in soil at planting. $Y = 4.20 - 1.73X + 0.24X^2$ ($R^2 = 0.991$; $P \leq 0.01$). B) Soil J2 population at harvest. $Y = 26916.5 - 8423.29X + 848.25X^2$ ($R^2 = 0.952$; $P \leq 0.05$). C) Root gall index, graded form 0 to 10 based on the percentage of roots galled. D) Pod gall index, graded from 0 to 10 based on the percentage of pods galled. E) Number of endospores attached to J2 at harvest. $\text{Log}_{10} Y = -1.34 + 0.438X$ ($r^2 = 0.986$; $P \leq 0.01$). F) Dry weight of foliage. G) Dry weight of pods. H) The percentage of J2 with attached endospores at harvest. $Y = 6.79X + 4.99X^2$ ($R^2 = 0.998$; $P \leq 0.01$).

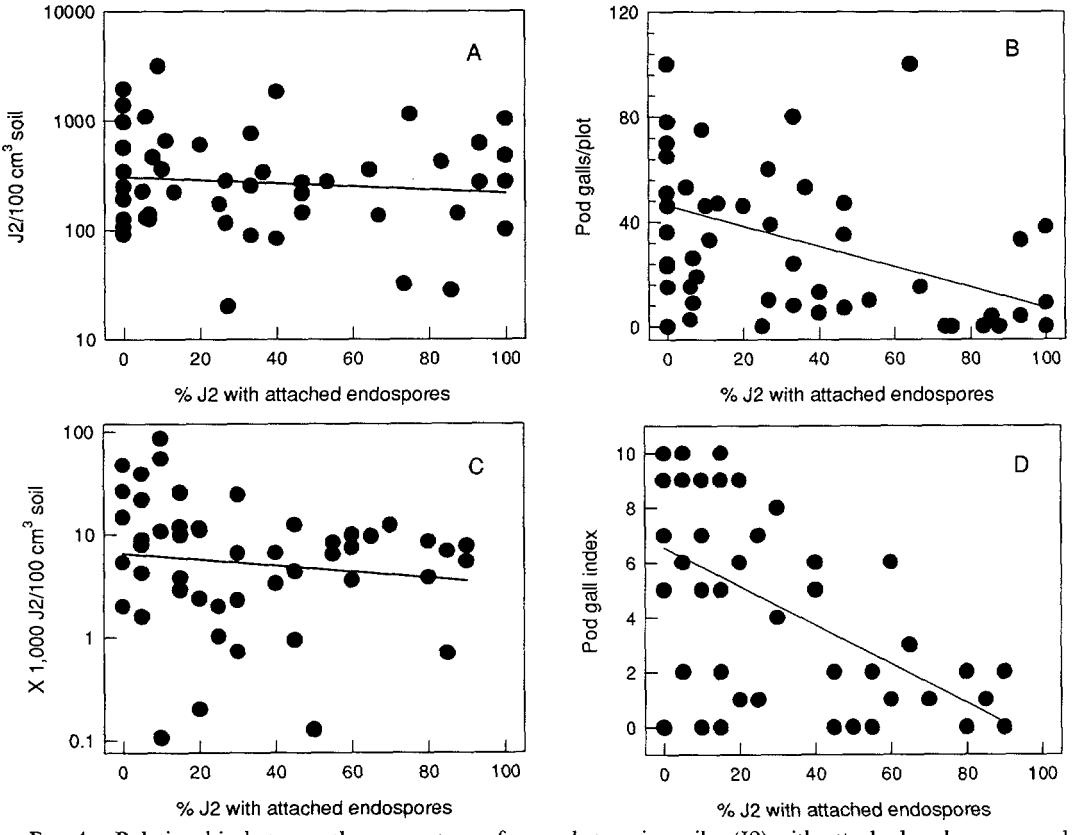

FIG. 4. Relationship between the percentage of second-stage juveniles (J2) with attached endospores and population changes of *Meloidogyne arenaria* race 1 on peanut. Data were collected from 49 field microplots in 1994–95 (one microplot in the control was contaminated with *P. penetrans* and excluded in the statistical analysis). A) Relationship between soil J2 population densities at harvest (1994) and the percentage of J2 with attached endospores (1994) at planting. The regression was not significant ($P > 0.05$). B) Regression of number of pod galls (1994) to the percentage of J2 with attached endospores (1994) at planting. $Y = 46.4 - 0.390X$, ($r = 0.40$; $P \leq 0.001$). C) Relationship between soil J2 population at harvest (1995) and the percentage of J2 with attached endospores (1994) at harvest. The regression was not significant ($P > 0.05$). D) Regression of pod gall index (1995) to the percentage of J2 with attached endospores (1994) at harvest. $Y = 6.56 - 0.071X$, ($r = 0.57$; $P \leq 0.001$).

nita. Treatment of *M. javanica* infested soil with *P. penetrans* alone (29) or in combination with aldicarb or carbofuran (5) reduced numbers of galls on tomato. Our results provide evidence that *P. penetrans* was responsible for the yield increase and gall reduction on peanut because they were related to the varied endospore inoculation levels.

The carry-over population densities of J2 in the soil in 1995 was high. It is well established that large numbers of *M. arenaria* can build up on peanut from preplant numbers at or below the detection limits of extraction methods (21,26,27). Densities of *M. arenaria* in soil increase exponentially during the peanut growing season (26). The highest final density of J2 in soil in our experiment reached 85,640 J2/100 cm^3 soil, which was much higher than a previously observed "carrying capacity" of 600–6,000 J2/100 cm^3 (21,25). It seems that *M. arenaria* has high reproduction potential on peanut in our deep sandy soil.

Pasteuria penetrans did not suppress the final population densities of J2 in soil. It is possible that the loss of fecundity of parasitized females can be compensated by increased reproduction of healthy females,

but the number of J2 in soil at a given time is dependent on so many factors that a generalization is difficult to make. Another factor that may be addressed is the exponential growth of *M. arenaria:* Pf = Pi * e^{rt} (26). In our experiment, e^{rt} was estimated approximately at 3,600. Any difference in Pi will be magnified 3,600-fold in Pf, which makes Pf unpredictable. We suspect that the overall lower final population densities of J2 in endospore-inoculated microplots in 1995 were due to the lowered Pi, which were caused by *P. penetrans* affecting the overwintering J2. Researchers have reported that *P. penetrans* reduces the motility of J2 in soil (6,9,20,29). In a petri dish experiment, kinesis of J2 was changed when J2 were attached with any number of endospores (Chen et al., unpubl.). Reduced motility probably leads to a high mortality of J2 in soil because movement apparently is essential to escape predators and unfavorable environmental conditions. Reduction of final J2 population in soil, however, was demonstrated in some experiments (8,10).

Although the final J2 population densities in soil are not significantly affected by *P. penetrans,* the population quality of J2 deteriorated. Percentage of J2 with attached endospores readily increased with the increasing endospore inoculation levels in soil. This suggests that *P. penetrans* may lead to the soil becoming suppressive to *M. arenaria* over the years (24). To date, *P. penetrans* has provided a continuous suppression on a mixed population of *M. incognita* and *M. javanica* in a 7-year monoculture of tobacco (13). Therefore, it appears that endospores of *P. penetrans* are persistent in soil and result in soil becoming suppressive over time (14,24).

Higher yields were obtained in all endospore inoculation levels than that of the control in our experiment. Reduction in galls was greater in plots treated with 10,000 and 100,000 endospores/g of soil than at 1,000 and 3,000 endospores/g of soil. This indicated that at least 10,000 endospores/g of soil may be needed to provide significant suppression of *M. arenaria.* This amount is equivalent to 2×10^{13} endospores/ha in the top 20 cm of soil. Our material contained approximately 7.9×10^7 endospores/g of root powder (7); thus, 250 kg of that powder would be required for broadcast treatment of 1-ha field. Endospore production has been reported as high as 1.2×10^9 (28) and 1.9×10^9 (16) endospores/g of root. If those techniques can be duplicated and similar numbers can be obtained for large-scale production, it would reduce the amount of root powder to 17 and 11 kg/ha, respectively. Should row treatment be used, the required amount of root powder could be reduced proportionately. When the effectiveness and longevity of the treatment over 2 years are considered, the application of *P. penetrans* has great potential in the near future for the biological control of *Meloidogyne* spp.

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