METHYL BROMIDE AND CHLOROPICRIN EFFECTS ON *PASTEURIA PENETRANS*.

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Pasteuria penetrans is an important biological control parasite of root-knot nematodes. It is a mycelial, endospore forming bacterial parasite and is known to cause soils to become suppressive to root-knot nematodes, *Meloidogyne* spp. The spores attach to the outside nematode body wall of the infective stage (second-stage juveniles [J2]). After the nematode penetrates a plant root and begins to feed the bacterium penetrates the nematode body wall. Once the bacterium is inside the nematode it begins to grow and develop. Shortly the nematode body becomes completely filled with spores. Each infected root-knot nematode female contains an average of 2.0 to 2.5 million spores, which are eventually released into the soil. Little is known about their long time survival in soil, or about the effects of pesticides on their survival. Our objective was to determine the effects of soil fumigants on *P. penetrans* and its infectivity of root-knot nematodes. The site chosen for the experiments was known to have a heavy infestation of both root-knot nematode and the spore forming bacterium.

Methods: Two experiments were conducted. In both trials the beds were mulched with polyethylene plastic and water applied via drip tubing. The plots were single rows with 3 ft. wide bed tops and 40 ft. long. In experiment 1 the treatments included methyl bromide (mbr) + 33% chloropicrin (pic), pic; two rates of 1,3-dichloropropene (1,3-D) + 16.5% pic; 1,3-D + 25% pic; 1,3-D + 35% pic; metam sodium (Na), and an untreated control, and in experiment 2 they included mbr + 33% pic, pic, and the untreated control. The treatments were arranged in a randomized complete block design with six replications per treatment. The fumigants were injected 10 inches deep with chisels spaced 12 inches apart. Metam Na was sprayed over the bed surface in water, and incorporated 4 to 6 inches deep with a rototiller. Tomato seedlings, cv. Agriset 761 were used in exp. 1, and tobacco cv. Coker 371-Gold and K-326 were used in exp. 2. Soil from each plot was taken 15 days after the chemicals were applied, placed in pots in the greenhouse where 2,400 untreated J2s were introduced to evaluate the infectivity and development of *P. penetrans* in the nematode. One 10-day-old seedling of dwarf cherry tomato, cv. Florida Petite was transplanted into each pot.

Results, Exp. 1: The gall rating was low in all plots regardless of treatment (Table 1). However, the number of J2 extracted from the soil taken from each plot was relatively high except for the mbr treated plots. These numbers are indicative of a soil that is suppressive to root-knot nematodes. Ninety-six percent of the J2 extracted from the field plots had spores of *P. penetrans* attached on their cuticle, with an average of 35 ± 25 spores/J2. There were little differences among the treatments in terms of number of fruit and weight of fruit, again suggesting that root-knot nematodes were having little impact on plant growth. When the

soil from each treated plot was taken to the laboratory and had healthy root-knot nematode J2 added we found greater galling on tomato when they were grown in soil retrieved from plots treated with mbr and pic than from plots treated with metam Na or the untreated control (Table 2). The number of J2 was lower in the untreated control than in the other treatments, except that the number was not different from that in soil treated with metam Na. Inversely, the percentage of nematode females with *P. penetrans* spores was greater in the untreated control and in the soil treated with metam Na than in the other treatments. No differences in number of J2 or in percentage of female nematodes with *P. penetrans* spores were observed among the other treatments. No differences in number of eggs per root system, root weight, or number of spores attached per J2 were observed among any of the treatments.

Exp. 2: In the tobacco plots the J2 were present at low population densities (1, 1, 2, 6, 9, and 21 J2/100 cm^3 of soil in only six of the 18 beds sampled). These numbers further decreased by the end of the season. *Pasteuria penetrans* spores were attached on most of the J2 from all plots. An average of 12 spores was attached to each juvenile in the control plots. The average of galls determined for 10 root systems of tobacco per plot was only 0.2 in the untreated controls, 0.02 in the mbr treated plots and 0.05 in the pic treatment. Few galls were produced in either cultivar of tobacco. Females were extracted from the few galled roots encountered in the field and assessed for the presence of *P. penetrans*. Twelve females were extracted from roots from each of two untreated control plots. The percentages of females with spores from these plots were 58% and 75%. Twenty females were extracted from one mbr treated plot and only one female had spores (5%). Sixteen females were extracted from a pic treated plot and none of them contained spores of *P. penetrans*.

DISCUSSION: Although nematodes were present in considerable numbers in most of the plots at the end of the tomato season, except in mbr treated plots, few galls developed in the tomato roots. The large numbers of spores attached to J2 in all treatments appeared to have reduced the penetration of J2 into the tomato roots. Most of the J2 extracted from field soil in this study each had more than 30 spores attached. The fumigants applied in this test did not affect spore attachment to the J2 under field conditions. Effects of chemicals on the development of the bacterium in its host nematode could not be evaluated because few females could be found in roots. Furthermore, the chemical affects on the nematode host could have changed indirectly the bacterial development. These results strongly indicate that the field site used in this study was suppressive to *M. arenaria* race 1, and that *P. penetrans* played an important role in the nematode suppression. Introducing untreated J2 into field soil with a natural population of *P. penetrans* that had been treated under field conditions and maintained in the greenhouse allowed the evaluation of the effects of the fumigants on spores of *P. penetrans* without interference of the effects of these chemicals on the nematode host. Greater gall indices in mbr 67-33 and pic treatments compared to untreated soil and metam Na treated soil indicate that these chemicals reduced attachment of spores to the introduced J2. Thus, more J2 with fewer spores attached would have been able to penetrate tomato roots in these treatments then in the untreated control, the latter of which had the greatest levels of attached spores. Although there were no differences in the number of eggs produced among any of the treatments, fewer J2 were detected in the untreated control than in any of the fumigant treatments, except the metam Na treatment. Percentages

of females with bacterial spores were greater in the untreated control and in the metam Na treated soil than in soil treated with other nematicides. Disease in *M. arenaria* females caused by *P. penetrans* was inversely related with the gall index and number of J2 in the soil in this study, which indicates that *P. penetrans* suppresses the nematode and that fumigant nematicides, except for metam Na, are detrimental to the development of *P. penetrans* in its nematode host. Data attained from these experiments indicated that pic alone or in combination with mbr was highly detrimental to *P. penetrans* because they inhibited spore formation within the female nematode. Formulations of 1,3-D + 16.5% pic, 1,3-D + 25% pic, and 1,3-D + 35% pic had moderate effects on the bacterium. The pic present in these formulations may have been responsible for the bactericide effect on *P. penetrans*. Metam Na was the only soil fumigant harmless to the bacterium and also was the only chemical not containing pic.

Table 1. Effects of methyl bromide + chloropicrin, chloropicrin alone, metam sodium, and formulations of 1,3-dichloropropene + chloropicrin on root knot of tomato, caused by *Meloidogyne arenaria*, in field soil naturally infested with *Pasteuria penetrans*.

Treatment	Gall index[a]	Number of J2[b]/100 cm^3 of soil	Number of endospores/J2	Number of tomato fruits/plot	Weight (kg) of tomato fruits/plot
Methyl bromide + 33% chloropicrin 350 lb/a	0.3 a[c]	1.3 b	41.5 a	141 ab	23.8 ab
Chloropicrin 335 lb/a	2.3 a	89.0 ab	39.5 a	108 b	17.0 b
Metam sodium 100 gal/a	1.2 a	106.0 ab	23.0 a	118 ab	19.3 ab
1,3-D + 16.5% chloropicrin at 35 gal/a	0.6 a	41.0 ab	44.4 a	145 ab	23.8 ab
1,3-D + 16.5% chloropicrin at 22 gal/a	1.1 a	60.0 ab	37.6 a	157 a	25.8 a
1,3-D + 25% chloropicrin 24 gal/a	1.1 a	21.0 ab	31.5 a	117 ab	18.5 ab
1,3-D + 35% chloropicrin 28 gal/a	1.7 a	134.0 a	40.6 a	140 ab	23.1 ab
Untreated control	1.3 a	25.5 ab	21.6 a	120 ab	21.0 ab

[a]Gall index was based on a scale of 0 to 10, in which 0 = 0 galls, 1 = 10% of roots with galls, 2 = 20% of roots with galls, 3 = 30% of roots with galls, and so on until 10 = 100% of roots with galls.
[b]J2 = second-stage juveniles of *M. arenaria*.
[c]Values are means of six replicates; means within columns followed by the same letter do not differ at $P \leq 0.05$ according to Duncan's multiple-range test.

Table 2. Effects of field applications of methyl bromide + chloropicrin, chloropicrin alone, metam sodium, and formulations of 1,3-dichloropropene + chloropicrin in greenhouse experiment 1on infectivity of *Pasteuria penetrans* to subsequently introduced *Meloidogyne arenaria* race 1, grown on tomato under greenhouse conditions.

Treatment[a]	Gall index[b]	Number of eggs/ root system	Root weight (g)	Number of J2[c]/ 100 cm^3 of soil	Number of endospores/J2	% females with *P. penetrans*
Methyl bromide + 33% chloropicrin 350 lb/a	8.3 a[d]	5,440 a	12.1 a	2,010 a	2.2 a	1.7 b
Chloropicrin 335 lb/a	8.3 a	5,240 a	12.1 a	1,930 a	1.6 a	0.0 b
Metam sodium 100 gal/a	5.5 b	3,890 a	8.7 a	850 bc	2.6 a	25.8 a
1,3-D + 16.5% chloropicrin 35 gal/a	6.5 ab	4,620 a	7.9 a	1,710 a	1.6 a	5.8 b
1,3-D + 16.5% chloropicrin 22 gal/a	6.0 ab	4,060 a	9.0 a	1,380 ab	1.7 a	7.5 b
1,3-D + 25% chloropicrin 24 gal/a	7.0 ab	5,010 a	10.3 a	1,580 ab	1.8 a	5.8 b
1,3-D + 35% chloropicrin 28 gal/a	7.5 ab	4,920 a	11.1 a	1,680 a	2.3 a	4.2 b
Untreated control	4.7 b	3,580 a	8.7 a	218 c	2.6 a	27.5 a

[a]Soil was treated with fumigants in a field naturally infested with *P. penetrans*; samples collected from the field were dried, infested with juveniles of *M. arenaria*, planted with tomato seedlings, and maintained for 75 days in the greenhouse.

[b]Gall index was based on a scale of 1 to 10 in which 0 = 0 galls, 1 = 10% of roots with galls, 2 = 20% of roots with galls, 3 = 30% of roots with galls, and so on until 10 = 100% of roots with galls.

[c]J2 = second-stage juveniles of *M. arenaria*.

[d]Values are means of six replicates; means within columns followed by the same letter do not differ at $P \leq 0.05$ according to Duncan's multiple-range test.