Improvement of Techniques for Determining Populations of Macroposthonia xenoplax in Dry Soil

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ABSTRACT

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Quantitative assays of soil samples from areas known to be heavily infested with *Macroposthonia xenoplax* consistently have indicated very low populations in samples that were collected after prolonged dry weather. Modifications of Jenkins' centrifugal-flotation procedure were tested to improve extraction of this nematode from dry field soil. Mixing dry samples from the field in water with a Waring Blendor just prior to extraction, or increase in the sucrose concentration in the flotation procedure, increased the numbers extracted. Best results were obtained, however, when the soil was moistened to 16-24% (v/w) 1-7 days before the routine procedure was done.

In South Carolina and other states, *Macroposthonia xenoplax* (Raski) Loof and DeGrisse (syn. *Criconemoides xenoplax* Raski) has been implicated in short life of peach (*Prunus persica* L.) trees (1, 2). This disease appears to be caused by a complex of interacting physical and biological factors, including nematodes (3, 5, 6). Thus control of *M. xenoplax* is a critical component of the control program for peach tree short life in South Carolina, and soil samples from peach orchards are assayed routinely for the presence of this nematode by using the centrifugal-flotation technique described by Jenkins (4). However, during dry periods of more than 7 days, numbers of *M. xenoplax* extracted by this technique decline drastically, so that few nematodes are recovered from infested soil.

Wehunt (*unpublished*) found that moistening dry soil 2 or 3 days prior to extraction resulted in extraction of higher numbers of *M. xenoplax*. Nonmoistened samples of the same soils, by comparison, usually yielded very few nematodes. Likewise, we have noted that numbers of *M. xenoplax* larvae and adults extracted increase within a short time after a dry period has ended (Lawrence and Zehr, *unpublished*). Since dry periods often coincide with times of the year when nematode assays are essential for control recommendations to peach growers, it is important to obtain an accurate assessment of populations of this nematode during dry periods.

Field observations indicated that low numbers extracted from dry soil probably are erroneous, perhaps because of deficiencies in extraction procedures. The objective of this research was to improve extraction methods so that samples collected from dry soil would be indicative of the actual nematode population.

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MATERIALS AND METHODS

Soil samples.—Soil was obtained from *M. xenoplax*-infested orchard sites with different soil types in three South Carolina counties. In each site, the soil had dried to low moisture levels following periods of hot, dry weather of more than 7 days. The top 5-cm of soil was removed, exposing feeder roots of the trees. Fifty liters of soil then were collected by shovel from around peach roots at depths of 5 to 15 cm, and transported to the laboratory in 100-liter plastic containers. Quantitative assays were made by Jenkins' centrifugal-flotation (4), using a Sorval GLC-2B centrifuge at 632 *g* and 1,750 rpm. Assays of the dry soil by this procedure showed fewer than six ring nematodes/100 cm^3 of soil. Moisture levels in the three types of soil (Lakeland fine sand, Faceville loamy sand, and Cecil loamy sand) averaged 0.5, 3.0, and 7.0% (w/w), respectively, when collected.

Soil moistening.—Tap water was thoroughly mixed in Lakeland fine sand and Faceville loamy sand samples until moisture levels reached 4, 8, 12, 16, and 20% (v/w). In Cecil loamy sand, which had a higher moisture level when collected, moisture levels tested were 7, 12, 16, 20, and 24%. Nonmoistened samples from each field were used as controls. The moistened soil was placed in sealed plastic bags and incubated 1, 2, 4, 7, 10, or 14 days at 5 and 25 C. Each moisture and temperature level was replicated six times.

Each sample was assayed at each time interval by the centrifugal-flotation procedure and nematodes were counted in a Fenwick Counting Slide (E. G. Millington, Herts, England).

Soil-particle dispersion.—Lakeland fine sand was utilized to study the effect of soil particle dispersion. One-hundred cm^3 samples of soil were dispersed in 200 ml of tap water with a Waring Blendor® (Model D5-7 Waring Products Corporation, New Hartford, Connecticut) at

low speed (8,000 rpm) for 15, 30, 45, or 60 sec prior to extraction. The experiment was repeated four times.
30, 45, or 60 sec prior to extraction. The experiment was repeated four times.

Soil residues from the standard centrifugal-flotation procedure were assayed again after dispersing in a blender. These residues included the larger soil particles that settle out after washing through the 850-μm screen, and the pellets that form during centrifugation. Six 100-cm^3 samples of the dry Lakeland fine sand were processed by the usual procedure and nematodes counted. The centrifuge pellets each were blended 15 sec and processed again by Jenkins' procedure.

Sucrose concentration.—Sucrose solutions of 908, 1,362, and 1,816 g/liter of tap water (2.60, 3.91, and 5.21 m, respectively) were compared with the standard, 454-g (1.30 m) concentration.

Sucrose concentrations also were tested in combination with soil particle dispersion and moistening, using the procedures described previously. Four samples were used with each procedure. When the soil dispersal procedure was used, samples were blended in a Waring Blendor for 15, 30, or 45 sec for Lakeland fine sand, Faceville loamy sand, and Cecil loamy sand, respectively. Preliminary tests indicated that these times were optimum for the three soil types. The sucrose concentration was 1,816 g/liter tap water, or the standard, 454-g/liter concentration. When soil was moistened, tap water was added to 16% soil moisture (v/w) 10 days before extraction.

Soil drying.—To determine the influence of different soil moisture levels on numbers of nematodes extracted, infested Lakeland soil (5.4% moisture) was dried in the laboratory. Each of six replicates consisted of 2,500 cm^3 soil in an uncovered aluminum container. Two containers of soil were dried simultaneously in a small incubator without air circulation. Moisture levels were determined and the nematodes were assayed after 5, 8, 9, 10, 11, 12, and 13 days.

In another experiment, infested Lakeland soil (5.4% moisture) was dried within 60 hours to 1.1% moisture in an incubator with air circulation. Dried soil was moistened to a level of 16%. Samples were stored in plastic bags at 5 C for 4 days, then nematodes were assayed. Soil was dried again for 7 days, and nematodes were assayed again. Soil was remoistened to 16% and held 6 days at 5 C before the final assay.

The rate of soil drying as related to nematode recovery was investigated with infested Lakeland fine sand (3.9% moisture). The initial population was 1,128 *M. xenoplax*/100 cm^3 soil. Four samples were dried at 25 C to 0.5% moisture within 18 hr, in an incubator with air circulation. Other samples were dried more slowly, requiring 40 hr or 13 days at 25 C to reach 0.5% soil moisture.

After 13 days, 100-cm^3 samples were withdrawn and extracted by four different procedures: (i) the standard centrifugal-flotation procedure; (ii) extraction by centrifugal-flotation after blending in water for 15 sec; (iii) extraction by centrifugal-flotation 4 days after adding tap water to 16% soil moisture; or (iv) increasing the sucrose solution to 1,816 g/liter (5.21 M).

RESULTS

Soil moistening.—Adding water to naturally dried soil from the field for 1 or more days before extraction resulted in extraction of much higher numbers of *Macroposthonia xenoplax* from all three soil types tested (Tables 1, 2). Highest numbers were consistently recovered at the highest moisture levels tested; i.e. 20% for Lakeland fine sand, 24% for Cecil loamy sand, and 20% for Faceville loamy sand. Assays of dry soil from the field yielded an average of six or fewer nematodes per 100 cm^3 soil. Highest numbers of nematodes usually were recovered from samples that had been moistened from 1 to 7 days (Table 1), but soil types differed. Highest numbers of nematodes were recovered from moistened

TABLE 1. Centrifugal-flotation extraction of *Macroposthonia xenoplax* as influenced by soil moisture, temperature, and time after adding tap water to air-dried, naturally infested Lakeland fine sand[a]

Temperature (C) and soil moisture[b] (%)	Days after water was added						Average
	1	2	4	7	10	14	
5 C							
Dry[c]	1 A[d]	5 A	3 A	3 A	2 A	1 A	3 A
4	294 BC	210 B	294 B	345 B	284 B	300 BC	288 B
8	334 BC	323 BC	318 BC	281 B	248 B	437 C	324 BC
12	396 C	282 BC	350 BC	324 B	252 B	236 BC	307 BC
16	372 C	370 C	441 C	355 B	296 B	174 AB	335 C
20	411 C	411 C	457 C	583 C	273 B	238 BC	396 C
25 C							
Dry	1 A	3 A	2 A	2 A	1 A	1 A	2 A
4	297 CD	245 B	223 B	164 B	126 B	115 A	195 B
8	286 CD	179 B	225 B	194 B	149 B	120 A	192 B
12	260 BC	201 B	187 B	143 AB	111 AB	89 A	165 B
16	353 DE	367 C	325 C	345 C	195 B	53 A	273 C
20	399 E	377 C	488 D	415 C	225 B	84 A	331 D

[a]Soil was collected near roots of 12-yr-old peach trees. Experiment began 14 days after soil was collected.
[b]Tap water was added to soil on vol/wt basis. Field capacity of soil was 9.3% at −1/10 bar.
[c]Air-dry when collected, with approximately 0.5% soil moisture.
[d]Numbers followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

Lakeland fine sand after 1 to 7 days, from Cecil loamy sand after 2 to 14 days, and from Faceville loamy sand after 1 or 2 days.

Moisture levels influenced significantly the number of nematodes extracted (Table 2). Generally, the number of nematodes extracted increased with each increment of soil moisture. The numbers extracted from Cecil loamy sand were not great unless the moisture level exceeded 16%. Numbers of nematodes extracted were affected more by moisture levels near saturation in the Cecil loamy sand than similar moisture levels in the other two soils. Saturation of soil resulted in soil compaction, and an increase of soil weight per 100 cm^3 of 14-40%. Thus, the higher numbers of nematodes extracted near saturation was partly a factor of more soil by weight per unit volume. In Lakeland fine sand, significant differences occurred between dry samples and those moistened to 4%, but differences between 4, 8, and 12% were not significant (Table 2).

Temperature seemed to have little influence on numbers extracted the first 7 days. After 7 days, however, numbers generally decreased in samples held at 25 C, but remained constant at 5 C, regardless of moisture level. After 14 days at 25 C, numbers in moistened Lakeland fine sand were not significantly higher than in dry samples.

Soil-particle dispersion.—Blending soil in water before extraction increased the number of *M. xenoplax* extracted from Lakeland fine sand. Preliminary tests showed that blending for 15 sec was best. From 100 cm^3 of soil, 199, 147, 105, and 135 nematodes were recovered after blending 15, 30, 45, and 60 sec, respectively. Initial tests of this soil showed levels of less than five when blending was not used.

Only a few nematodes were present in the coarse soil fraction that settled out during the extraction procedure, but many nematodes were present in the finer soil fraction collected during centrifugation in the sucrose solution. When the soil pellet was blended and extracted again, these nematodes remained in suspension. Nematodes thus extracted were of normal appearance. Although only eight *M. xenoplax*/100 cm^3 soil were extracted with the standard procedure, dispersing the pellets in a blender before extraction yielded an average of 138 nematodes.

Effect of sucrose concentration.—Processing samples of Lakeland fine sand with sucrose concentrations greater than those used in Jenkins' procedure resulted in extraction of more ring nematodes. Using the standard concentration of 454 g sucrose in 1 liter of water, 25

TABLE 2. Centrifugal-flotation extraction of *Macroposthonia xenoplax* as influenced by percent soil moisture after adding tap water to air-dried, naturally infested soils[a]

Soil moisture (%)	Nematodes per 100 cm^3 of: Cecil loamy sand[b] (no.)	Faceville loamy sand[c] (no.)	Lakeland fine sand[d] (no.)
1	...	...	2 A
3-4	...	1 A	247 B
7-8	1 A	132 B	261 B
12	20 B	186 C	250 B
16	37 C	314 D	332 C
20	93 D	375 E	391 D
24	192 E	...	...

[a]Numbers/100-cm^3 soil at each moisture level.
[b]Field capacity was 15.5% at −1/10 bar.
[c]Field capacity was 11.9% at −1/10 bar.
[d]Field capacity was 9.3% at −1/10 bar.

TABLE 3. Effect of variations of Jenkins' procedure on numbers of *Macroposthonia xenoplax* from Lakeland fine sand, Cecil loamy sand, and Faceville loamy sand

Treatment	Nematodes per 100 cm^3 of: Lakeland fine sand (no.)	Cecil loamy sand (no.)	Faceville loamy sand (no.)
Control (no variation)	5 A[d]	2 A	0 A
Soil blended[a]	26 A	74 A	59 AB
Increased (>) sucrose[b]	239 BCD	486 C	129 BC
Soil moistened[c]	269 CD	218 B	228 D
Blended + >sucrose	186 BC	466 C	156 BCD
Moistened + >sucrose	171 B	546 C	320 E
Moistened + blended	275 D	293 B	197 CD
Blended + moistened + >sucrose	157 B	566 C	191 CD

[a]Soil was blended in 200 ml water 15 sec for Lakeland fine sand, 30 sec for Faceville loamy sand, and 45 sec for Cecil loamy sand.
[b]Concentration of sucrose solution was increased from 454 to 1,816 grams/liter water.
[c]Extraction was done 10 days after samples were moistened to 16%.
[d]Numbers followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

TABLE 4. Numbers of *Macroposthonia xenoplax* recovered from Lakeland fine sand dried to various moisture levels[a]

Moisture (%)	*M. xenoplax* (no./100 cm^3 soil)
4.4 − 4.6	240
3.7 − 3.9	175
2.9 − 3.4	131
2.4 − 2.7	91
2.0 − 2.1	11
1.1 − 1.5	5

[a]Temperature was 25 C over a period of 12-13 days. Average moisture level was 5.4%, and nematode counts averaged 337/100 cm^3 soil at the beginning of the experiment.

TABLE 5. Effect of repeated drying and rewetting of soil[a] on numbers of *Macroposthonia xenoplax*/100 cm^3 soil extracted from Lakeland fine sand

Procedure	*M. xenoplax* (no./100 cm^3 soil)
Initial population	244
Dried at 25 C to 1.6% moisture	3
Moistened[b] to 16%	95
Redried at 25 C to 0.7% moisture	1
Remoistened[c] to 16%	4

[a]Soil moisture was 4.2% at beginning of experiment.
[b]Tap water was added to soil 4 days prior to extraction, to a level of 16% moisture.
[c]Soil rewetted to 16% 6 days prior to extraction.

nematodes/100 cm^3 soil were extracted, but 88, 162, and 240 nematodes were extracted when 908, 1,262, and 1,816 g sucrose/liter (2.60, 3.91, and 5.21 M), respectively, were used.

Little additional benefit resulted when two or all three variations of the extraction procedure were used in sequence (Table 3). However, in Cecil loamy sand high sucrose concentration and soil moisture together increased the number of nematodes extracted.

Effects of drying.—Numbers of *M. xenoplax* extracted from field soil decreased steadily as moisture levels declined from 4.6% to 1.5% (Table 4) over a period of 12 to 13 days. Soil moisture levels were positively correlated ($r = 0.8415$) with the number of nematodes extracted. After tap water was added to soil dried in the laboratory, the numbers extracted increased (Table 5), but not to the level found before drying commenced. Repeated drying and moistening of such soil resulted in extraction of very few nematodes. Drying soil over a 13-day period had the same effect as an 18-hr period.

DISCUSSION

Results of these experiments show that soil moisture levels have important effects on the numbers of *M. xenoplax* extracted when the centrifugal-flotation procedure is used. Critical soil moisture levels differ with soil type. Under South Carolina conditions, a period of 7 to 10 days without rainfall during summer and fall months may be sufficient to interfere with nematode extraction from soil at the 15-cm depth.

Dry conditions in the soil appear to be injurious to ring nematodes, as shown by decreased numbers after repeated drying. However, the primary influence of dry soil conditions in the field is on the extraction procedure. In dry soils, the nematodes do not remain suspended in the sucrose solution at the concentration customarily used. Thus, results obtained from dry soils when using the centrifugal-flotation procedure may be erroneous due to limitations of the technique.

Our experiments show that the problems encountered in dry soils can be corrected, by (i) moistening the soil prior to extraction; (ii) dispersing the soil particles in water in a blender prior to extraction, or (iii) increasing the amount of sucrose in the flotation procedure to obtain a higher specific gravity. Of these, moistening the soil at least 24 hr before extraction was most consistent in the three soil types used. The soil moistening technique now is used routinely in the Clemson University Plant Problem Clinic for assays of soil samples that were collected under dry conditions.

Based on these experiments the most consistently effective procedure was to moisten dry soils to near saturation, and store them for 1 to 7 days before extraction. Temperature during storage did not affect numbers extracted during this time period. With certain soil types, however, increasing the sucrose concentration may be equally or more effective than moistening the soil before extraction.

LITERATURE CITED

1. CHITWOOD, B. G. 1949. Ring nematodes (Criconematinae). A possible factor in decline and replanting problems of peach orchards. Proc. Helminthol. Soc. Wash. 16:6-7.
2. DE VAY, J. E., B. F. LOWNSBERY, W. H. ENGLISH, and H. LEMBRIGHT. 1967. Activity of soil fumigation in relation to increased growth response and control of decline and bacterial canker in trees of Prunus persica. Phytopathology 57:809 (Abstr.).
3. HENDRIX, F. F., JR., and W. M. POWELL. 1970. Control of root pathogens in peach decline sites. Phytopathology 60:16-19.
4. JENKINS, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48:692.
5. NESMITH, W. C., and W. M. DOWLER. 1975. Soil fumigation and fall pruning related to peach tree short life. Phytopathology 65:277-280.
6. ZEHR, E. I., R. W. MILLER, and F. H. SMITH. 1976. Soil fumigation and peach rootstocks for protection against peach tree short life. Phytopathology 66:689-694.