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Seasonal Population Fluctuation of *Xiphinema americanum* and *X. rivesi* in New York and Pennsylvania Orchards[1]

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Abstract: The population fluctuation and composition of *Xiphinema americanum* (sensu stricto) and *X. rivesi* were studied in a New York apple orchard (only *X. americanum* present), a Pennsylvania apple orchard (both *X. americanum* and *X. rivesi* present), and a Pennsylvania peach orchard (*X. americanum, X. rivesi,* and *X. californicum* present). Few clear trends in population fluctuation or composition were observed. The adult female was the predominant stage in most sample periods, and the reproductive period was limited to late spring and early summer. Only a few of the females at any sample period were gravid. All stages were present throughout the year, and all stages overwintered. Eggs in soil were not monitored. In the Pennsylvania apple orchard, *X. americanum* and *X. rivesi* were easily separated by morphological characteristics; however, the two species did not display differences in population structure or composition. The predominance of adults, the relatively low reproductive rates, and the association of these species with stable habitats suggest that the life strategies of *X. americanum* and *X. rivesi* are K-selected as opposed to r-selected.

Key words: apple, dagger nematodes, *Malus,* Nematoda, *Prunus, Xiphinema americanum, X. rivesi.*

Seasonal fluctuations in population density of *Xiphinema americanum* (sensu lato) have been described for several hosts and locations (6,8,11,18,19,23). Recently, *X. americanum* (sensu lato) was divided into more than 23 species (12,14–16), and the distribution of *X. americanum* (sensu stricto) was said to be limited to the eastern United States (14). Therefore, the identification of species in earlier reports is not clear (6,11,18,19,23).

Species belonging to *Xiphinema americanum* (sensu lato) are recognized solely on the basis of morphological characteristics, and little is known of their biology, ecology, and physiology. Of these species, *X. americanum* Cobb (sensu stricto) (hereafter referred to as *X. americanum*) and *X. rivesi* Dalmasso occur commonly in New York and Pennsylvania fruit orchards (9; M. B. Harrison, unpubl.). Both species are considered serious pathogens because they vector tomato ringspot virus (2) and because nonviruliferous specimens may also incite disease (3).

The purpose of this study was to describe seasonal changes in population levels of *Xiphinema* spp. in a New York apple orchard, a Pennsylvania apple orchard, and a Pennsylvania peach orchard. Preliminary samples indicated that the New York orchard contained *X. americanum* and that the Pennsylvania orchards contained both *X. americanum* and *X. rivesi.*

Materials and Methods

Soil samples were collected from around apple trees (Red Rome on MM.106) grown in bottomless fiberglass microplots (0.5 m d by 0.6 m deep) containing orchard soil infested with *Xiphinema* sp. The plots, located at Cornell University, Ithaca, New York, were planted in 1978. The *Xiphinema*-infested, sandy loam soil, obtained from an apple orchard in Monroe County, New York, also contained low population levels (< 50/100 cm^3 soil) of *Pratylenchus* sp. and *Criconemella* sp. Soil samples were also collected from an apple (Red Delicious on MM.106) and a peach (Loring on Halford) orchard in Pennsylvania. The apple trees were planted at the Pennsylvania State University orchard in Biglerville (gravelly loam soil) in 1976, and the peach trees were planted at the Pennsylvania State Univer-

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TABLE 1. Characteristics of soils.

Characteristic	Orchard		
	NY-apple	PA-apple	PA-peach
Sand (%)	56	47	43
Silt (%)	32	32	35
Clay (%)	12	21	22
pH	6.7	6.1	6.7
Organic matter (%)	2.2	1.4	2.7
CEC (meq/100 g)	9.1	8.5	11.8
NO_3 (ppm)	57	48	22
P (ppm)	238	118	265
K (ppm)	109	168	238
Mg (ppm)	192	84	108
Ca (ppm)	1,040	700	1,640
Saturation %			
K	3.0	5.0	5.1
Mg	17.7	8.7	8.1
Ca	57.3	40.7	69.7

Texture analysis was determined by the hydrometer method. Other determinations were performed by Soil and Forage Testing, Merkle Laboratory, Pennsylvania State University.

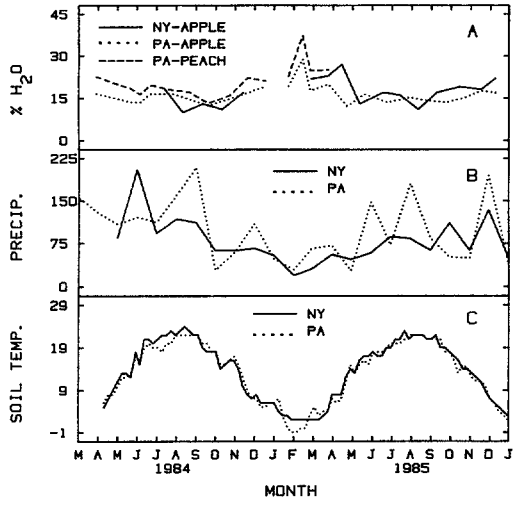

FIG. 1. Soil moisture, precipitation, and soil temperature in New York and Pennsylvania orchards. A) Soil moisture (%) in soil samples collected monthly. B) Monthly precipitation in mm. C) Soil temperature (Celsius) at 20 cm.

sity orchard in Arendtsville (gravelly loam soil) in 1979. In addition to *Xiphinema* spp., both Pennsylvania soils contained low population levels (< 25/100 cm^3 soil) of *Hoplolaimus galeatus, Pratylenchus* sp., and *Helicotylenchus digonicus.* Textural and chemical components of the three orchard soils were characterized (Table 1). A zone around each tree was kept weed-free by shallow cultivation (New York) or by herbicides (Pennsylvania). Standard fertilization and foliar pest control procedures were followed. Trees were irrigated during dry periods in New York but not in Pennsylvania.

The same 10 NY-apple, 12 PA-apple, or 8 PA-peach trees were sampled monthly except when the soil was frozen. One sample, about 80 cm^3 soil, was collected from each tree with an Oakfield tube (2 × 24 cm). Sampling was limited to 24 cm because penetration more than 24 cm into the gravelly Pennsylvania soils was difficult. The samples were combined, screened (1-cm-d pore size), and mixed gently. Nematodes were extracted from five 100-cm^3 subsamples from each orchard. Each subsample was incubated 1–2 hours in a 1-liter beaker containing 500 ml distilled water, the volume was increased to 800 ml with distilled water, and the suspension was poured rapidly between two 1-liter beakers 10 times. The suspension was allowed to settle 20 seconds, and the supernatant was then gently poured through a fine sieve (38-μm-d pore size). Mixing and sieving were repeated with each subsample. The material collected on the sieve was washed onto a Baermann funnel (20 cm d) containing about 500 ml distilled water. The funnels were incubated at room temperature (19–25 C) and the nematodes collected after 20, 44, and 68 hours. After collection, nematodes were counted and suspensions were combined, placed in a 45 C oven for 30 minutes, and mixed with an equal volume of warm 7.5% formalin. The percentage of soil moisture was determined in an additional subsample (Fig. 1A). Additional PA-apple subsamples were soaked, agitated, and sieved as described above and then processed by centrifugation (5 minutes in water at 1,800 rpm [600 *g*] followed by 1 minute in sucrose [454 g/liter solution] at 1,000 rpm [200 *g*]). Extraction always began within 4 hours of sample collection.

After fixation, 200 randomly selected *Xiphinema* spp. were examined at 400 × with a light microscope; if fewer than 200 specimens were present, all specimens were examined. Coverslips were supported by glass

rods (New York) or adhesive tape (Pennsylvania). Each nematode was categorized as follows: adult male, adult female, first-stage juvenile (= J1), second-stage or third-stage juvenile (= J2–3), or fourth-stage juvenile (= J4). Total stylet length (odontostyle plus odontophore), tail length and width, and head and tail shape were determined for each adult female. Head shape was rated from 1 to 3 (1 = lip region offset, 3 = lip region not offset, 2 = intermediate) as was tail shape (1 = pointed, 3 = rounded, 2 = intermediate). Stylet length, tail shape, and head shape are important characteristics distinguishing *X. americanum* and *X. rivesi* (15,25). Periodic examination of specimens indicated that, except for a low number of *X. californicum* in the PA-peach orchard, *X. americanum* and *X. rivesi* were the only *Xiphinema* spp. present. Total stylet length was also determined for each J1. Although length of odontostyle is considered especially useful for separating species (13), we measured the length of the total stylet (odontostyle plus odontophore) because a large number of nematodes were being measured and the total stylet was easier to measure than the odontostyle. Juvenile life stages were identified as follows: In J1, the replacement odontostyle overlaps the odontophore (4); in J2–3 and 4, the replacement odontostyle is positioned posterior to the current odontophore (4); in J4, immature reproductive organs are evident, and J4 are larger than J1, 2, and 3.

Extraction efficiency was determined in an early test using field soil infested with *X. americanum* (85%) and *X. rivesi* (15%) maintained in a 15-liter pot in the greenhouse with an orchard sod as the host. A 1.5-liter sample of soil was removed from the pot, screened (1-cm-d pore size), mixed, and divided into twelve 100-cm^3 subsamples. The subsamples were soaked and sieved as described for orchard samples. Half the subsamples were placed on the Baermann funnel for 20 hours and the remainder centrifuged as described for orchard samples. Total numbers and numbers of each stage of *Xiphinema* spp.

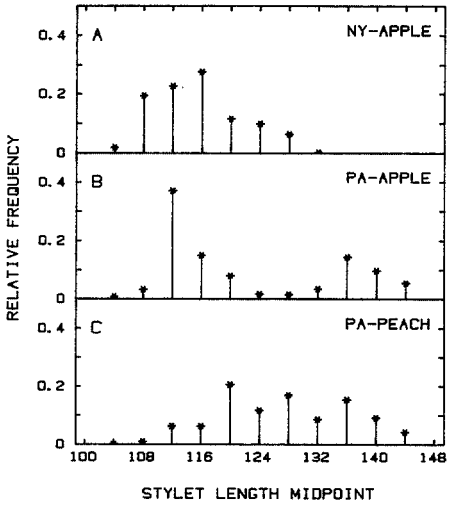

FIG. 2. Relative frequency distributions of stylet lengths (odontophore + odontostyle in μm) for adult female *Xiphinema* spp. in three orchards.

recovered from each sample were determined. Extracted and counted nematodes from each subsample were added to 1-liter beakers containing 100 cm^3 autoclaved PA-apple orchard soil plus 800 ml distilled water, mixed, sieved, and centrifuged as described for orchard samples. After centrifugation, the total number and stages of *Xiphinema* spp. were determined. This experiment was performed four times.

In a second test of extraction efficiency, soil samples from the PA-apple orchard were processed by Baermann funnel as described earlier. After 20 hours, the recovered *Xiphinema* spp. were counted and added to 1-liter beakers (20–50 *Xiphinema* spp. per beaker) containing 100 cm^3 autoclaved PA-apple orchard soil in 800 ml distilled water; the suspension was mixed, sieved, and placed on funnels, and the number of *Xiphinema* spp. recovered after 68 hours was determined. Control funnels contained autoclaved soil to which nematodes were not added. This experiment was repeated twice, with three replications per test.

Species identifications were confirmed by Dr. A. M. Golden. Voucher specimens from NY-apple, PA-apple, and PA-peach orchards were deposited with the USDA Nematode Collection, Beltsville, Mary-

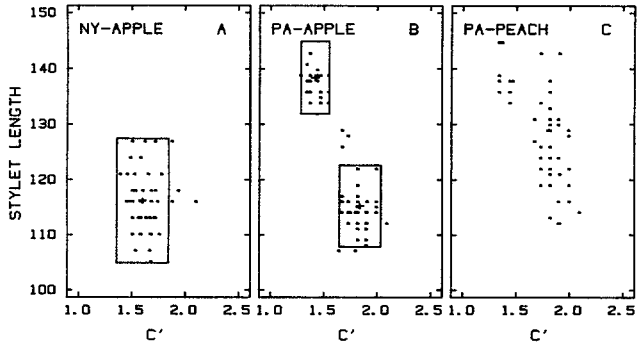

FIG. 3. Relationship between c' (tail length/anal body diameter) and stylet length (μm) for female *Xiphinema* spp. in three orchards. Fifty randomly selected observations were plotted per orchard. A) Rectangle includes 94% of all specimens (*X. americanum*) measured. B) Upper and lower rectangles include 96% and 92% of the specimens identified as *X. rivesi* and *X. americanum*, respectively; only 7% of the observations occurred outside the rectangles in B. C) See text for explanation.

land. Monthly precipitation was recorded (Fig. 1B). Mean soil temperatures 20 cm deep (weekly minimum plus maximum divided by 2) were calculated (Fig. 1C). In Pennsylvania, the temperature probe was located in the herbicide strip adjacent to a peach tree and approximately 1 km from the apple orchard and 6 km from the peach orchard. In New York, the temperature probe was located in sod at the Cornell University Weather Station, about 1 km from the apple orchard. In certain figures, the low variances associated with stylet lengths and c' values were emphasized by using ± 2 standard deviations or ± 2 standard errors of the mean.

RESULTS

Species identification: The relative frequency distribution of stylet lengths of females from the NY-apple orchard suggests that one population was present (Fig. 2A). All females from NY-apple had an offset lip region and 84% had c' values (tail length/anal body diameter) greater than 1.50 ($\bar{x} \pm SE = 1.60 \pm 0.14$). A relationship between stylet length and c' was evident (Fig. 3A). Although only 50 random observations are shown, 92% of all females measured (1,542) were within ± 2 SD of the mean for stylet length and c' value. The mean stylet length of this population was relatively constant (Fig. 4) as were c' values (data not shown). Representative specimens from NY-apple were identified by A. M. Golden as *X. americanum.*

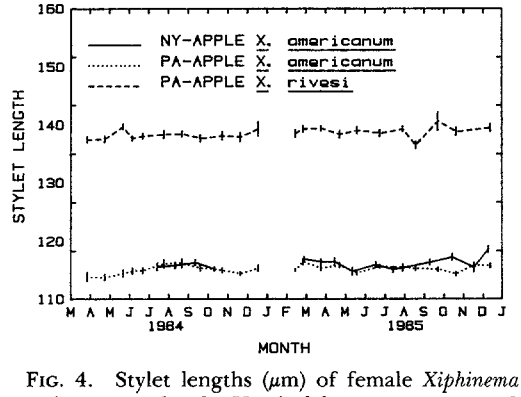

FIG. 4. Stylet lengths (μm) of female *Xiphinema* spp. in two orchards. Vertical bars represent ± 2 SEM.

The frequency distribution of stylet lengths of adult females from PA-apple suggests that two populations were present (Fig. 2B). Stylet lengths, c' values, and head and tail ratings were strongly correlated. Over 97% of females with stylets longer than 130 μm had c' values less than 1.55 and head and tail ratings of 3 (Table 2). Of specimens with stylets equal to or less than 130 μm, over 98% had c' values equal to or greater than 1.55, and head and tail ratings of 1 (Table 2). Females with shorter stylets, offset lip regions, and pointed tails were identified as *X. americanum* by A. M. Golden, whereas females with longer stylets, nonoffset lip regions, and rounded tails were identified as *X. rivesi.* In analyzing the data base of 2,032 adult females from PA-apple, specimens were considered to be *X.*

TABLE 2. Relationship between stylet length, c′ value, head shape, and tail shape for *Xiphinema* spp. in a Pennsylvania apple orchard.

Variable	Ratio or rating	Females (%) Stylet ≤ 130 μm	Stylet > 130 μm
c′	≥ 1.55	99.7	0.9
	< 1.55	0.3	99.1
Head shape†	1	98.2	0.3
	2	1.5	2.1
	3	0.3	97.6
Tail shape‡	1	98.3	0.3
	2	1.3	1.2
	3	0.4	98.5

A total of 2,030 females were examined.
† Head rating: 1 = lip offset; 3 = lip not offset; 2 = intermediate.
‡ Tail rating: 1 = pointed; 3 = rounded; 2 = intermediate.

rivesi if stylet length was ≥ 129 μm, c′ < 1.55, and head and tail ratings were 2 or 3. Similarly, specimens were considered to be *X. americanum* if stylet length was ≤ 134 μm, c′ ≥ 1.55, and head and tail ratings were 1 or 2. The overlap of minimum and maximum stylet lengths for *X. rivesi* and *X. americanum* was based on previous reports. Only 4% of the females had stylet lengths between 129 and 134 μm, and 0.5% of all adult females could not be classified as *X. americanum* or *X. rivesi* based on these criteria. Stylet lengths of adult female *X. americanum* ($\bar{x}$ = 115 μm) and *X. rivesi* ($\bar{x}$ = 139 μm) in PA-apple were relatively constant (Fig. 4) as were c′ values (data not shown). Stylet length and c′ for 50 randomly selected females from PA-apple were related (Fig. 3B). The upper rectangle (± 2 SD for stylet length and c′) in Figure 3B includes 92% of the specimens identified as *X. rivesi,* and the lower rectangle includes 96% of the specimens identified as *X. americanum.* Only 7% of the 2,032 observations fell outside of the rectangles.

The frequency distributions of stylet lengths of adult females from PA-peach and PA-apple orchards differed (Fig. 2). Early in the study, only *X. americanum* and *X. rivesi* were detected in PA-peach; the *X. rivesi* from PA-peach appeared similar to the *X. rivesi* from PA-apple, but the *X. americanum* had longer stylets ($\bar{x}$ = 124 μm).

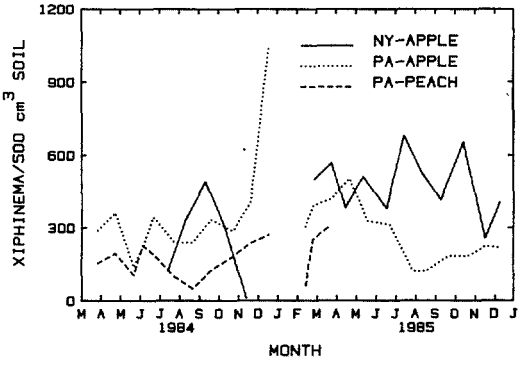

FIG. 5. Population densities of *Xiphinema* spp. in three orchards.

Near the end of the first year of sampling, 15 specimens with long stylets (132–136 μm) and markedly offset lip regions were observed and identified as *X. californicum* Lamberti and Bleve-Zacheo by A. M. Golden. Because the measurements and rating systems did not permit separation of *X. americanum* with long stylets and *X. californicum* in the data base, the data from PA-peach were not classified by species and sampling was terminated after 1 year. The relationship between stylet length and c′ for 50 randomly selected females from PA-peach suggests that two or three populations were present (Fig. 3C).

Population fluctuation and composition: In NY-apple, population levels increased during summer 1984 and then dropped sharply in fall, but the overwintering population level was higher than the prewinter population level. Population levels did not decline dramatically in late fall 1985 (Fig. 5). In PA-apple, population levels increased sharply in fall 1984, declined over winter, increased in spring 1985, decreased in summer, and rose somewhat in fall 1985 (Fig. 5). In PA-peach, population levels declined in summer 1984, increased in fall, declined over winter, and increased in spring 1985 (Fig. 5). Numbers of female *X. americanum* and *X. rivesi* in PA-apple varied through the year but followed similar trends (Fig. 6).

Males were rare, with only 8 among the 9,130 specimens examined at 400×. Females predominated at all sites at most sample periods (Fig. 7). The percentage of

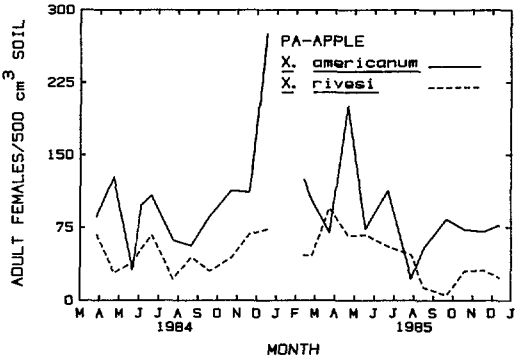

FIG. 6. Levels of females of *Xiphinema americanum* and *X. rivesi* in a Pennsylvania apple orchard.

gravid females (based on total number of females) never exceeded 20% and peaked in May–July at all sites; few gravid females were found in fall or early spring (Fig. 8). In PA-apple, gravid *X. americanum* and *X. rivesi* occurred at the same time (Fig. 8B).

All sites had J1 at all sampling periods (Fig. 7). In PA-apple and peach, J1 peaked 1–2 months after gravid females peaked (Fig. 7B, C), but J1 peaks in NY-apple did not necessarily follow peaks in gravid females (Fig. 7A). To identify J1 from PA-apple as *X. americanum* or *X. rivesi*, the stylet lengths of 25 J1 from three pure populations cultured on Sudan grass in the greenhouse were determined. The ranges of stylet lengths of 25 *X. rivesi* J1 originally obtained from a Pennsylvania vineyard and New York apple orchard were 75–80 μm and 77–82 μm, respectively. Stylet lengths of 25 *X. americanum* J1 from a New York apple orchard ranged from 66 to 71 μm. Thus, J1 with stylets ≥ 74 μm were considered *X. rivesi* and J1 with stylets < 74 μm were considered *X. americanum*. Numbers of J1 (*X. americanum* and *X. rivesi*) in PA-apple varied through the year but followed similar trends (Fig. 9). The proportion of J4 in the population tended to increase in fall and decrease in spring (Fig. 7), but levels of J2–3 displayed no consistent trends and were excluded from figures.

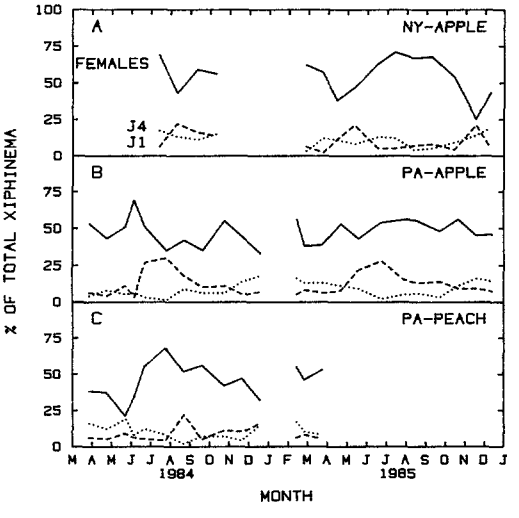

FIG. 7. Relative numbers of females, first-stage juveniles (J1), and fourth-stage juveniles (J4) in three orchards. In Figures 7 and 8, percentages from November 1984 NY-apple were excluded because only nine specimens were observed.

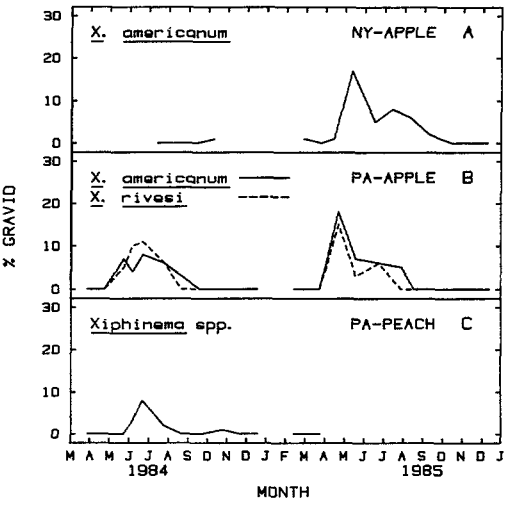

FIG. 8. Percentage gravid females (no. gravid females × 100/no. females) of *Xiphinema* spp. in three orchards.

Extraction efficiency: Extraction of nematodes from PA-apple soil by sieving–Baermann funnel provided higher population estimates than extraction by sieving–centrifugation (Fig. 10). The ratio of nematodes extracted by sieving–Baermann funnel vs. sieving–centrifugation was greater in fall, winter, and spring and less in summer. The mean ratio of nematodes extracted by funnel vs. centrifuge for all sample periods in PA-apple was 2.0. The number of nematodes extracted by funnel was negatively correlated ($r = -0.53$, $P =$

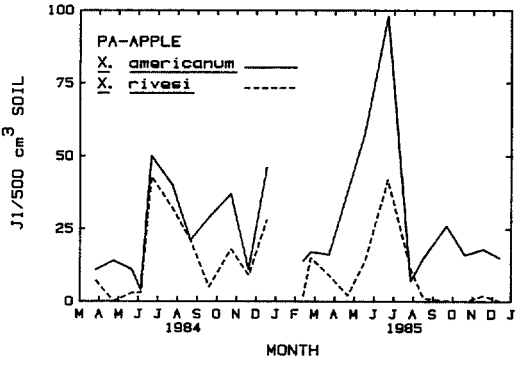

FIG. 9. Levels of first-stage juveniles (J1) of *Xiphinema americanum* and *X. rivesi* in a Pennsylvania apple orchard.

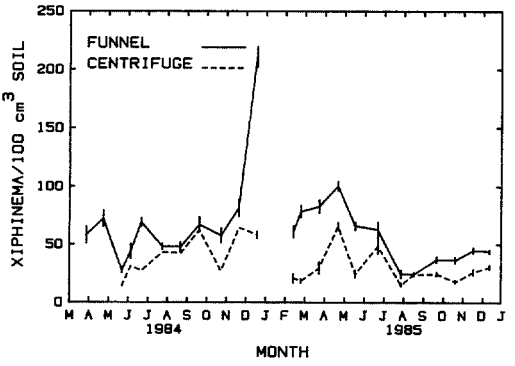

FIG. 10. Numbers of *Xiphinema* spp. extracted by Baermann funnel or centrifugation. For Baermann funnel, each value is the mean of five replications. For centrifugation, each value is the mean of one replication (May–November 1984) or three replications (December 1984–December 1985). Vertical bars represent ± SEM.

0.03) with field soil temperature. The percentage of *Xiphinema* extracted from PA-apple after 1 or 2 days on the Baermann funnel was variable, but most nematodes were recovered after 1 day (Fig. 11).

In the first extraction efficiency test, the number of nematodes extracted by centrifugation in the second extraction was greatly influenced by the method used to obtain nematodes in the first extraction (Table 3). Extraction efficiency by centrifugation was 15 or 30% depending on whether nematodes were obtained by funnel or centrifuge, respectively. Because the mean relative efficiency of funnel to centrifuge was 2.0 (see previous paragraph and Fig. 10), the extraction efficiency of sieving–Baermann funnel based on these data was estimated to be 30–60%. Stage-specific differences in extraction efficiency were not detected; the population composition of extracted samples with known composition did not change when the samples were sieved and centrifuged a second time (Table 3). Both extraction methods (sieving–centrifugation and sieving–Baermann funnel) provided similar estimates of population composition (Table 3). In the second extraction efficiency test, known numbers of *Xiphinema* spp. obtained by Baermann funnel were added to Baermann funnels and recovery was determined after 64 hours. The extraction efficiency in this case was 66 ± 15% ($\bar{x}$ ± SD). Neither species nor stage was determined before or after extraction in this test.

DISCUSSION

The present study of *X. americanum* (sensu stricto) and *X. rivesi* indicated few clear trends in population fluctuation and composition. In Pennsylvania fruit orchards, past experience suggested that population levels were usually highest in spring and fall and lowest in summer (L. B. Forer, pers. comm.). This observation was generally supported by the data from PA-apple and peach but not from NY-apple. Trees in Pennsylvania were not irrigated, but trees in New York were. The female was the predominant stage in most sample periods at all three sites, and the repro-

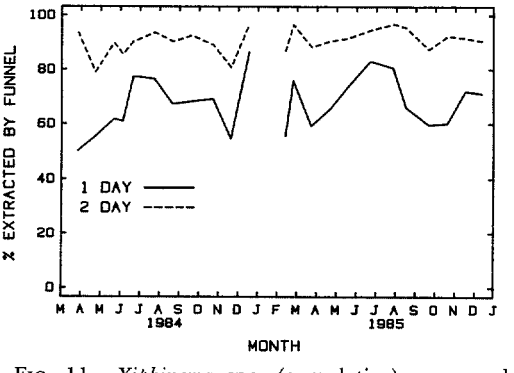

FIG. 11. *Xiphinema* spp. (cumulative) recovered from Baermann funnels after 1 day (20 hours) or 2 days (44 hours) incubation. Numbers obtained after 3 days (68 hours) incubation were considered to represent 100% of the nematodes.

TABLE 3. Extraction efficiency of sieving–centrifugation as influenced by source of *Xiphinema* spp.

Extraction	Source of nemas	Extract. method	*Xiphinema*/100 cm³	Percent of total		
				Adults	J2–4	J1
1	Soil	Centrifuge	124 ± 31	48 ± 6	33 ± 3	19 ± 3
1	Soil	Funnel	123 ± 16	54 ± 6	33 ± 5	14 ± 2
2	Centrifuge	Centrifuge	37 ± 10	49 ± 4	32 ± 3	19 ± 3
2	Funnel	Centrifuge	18 ± 5	53 ± 5	30 ± 4	16 ± 2

Field soil containing *X. americanum* (85%) and *X. rivesi* (15%) was extracted by sieving–centrifugation or sieving–Baermann funnel (Extraction 1). Funnels were tapped after 20 hours. Extracted nematodes were counted and again extracted by sieving–centrifugation (Extraction 2).

Each value is the mean ± SEM of four replicates.

ductive period was largely restricted to late spring and early summer. Few females were gravid at any sample period. All juvenile stages (J1, J2–3, J4) were present throughout the year, and the proportion of each stage was relatively stable; however, peaks in J1 were observed after peaks in gravid females in Pennsylvania. All vermiform stages appeared to overwinter, but eggs in soil were not monitored. The predominance of females was not due to differences in stage-specific extraction efficiency (Table 3).

There are several possible explanations why stronger and more regular trends in population fluctuation and composition were not detected. First, variation due to spatial distribution of nematodes, level of nematode activity, and extraction conditions may have masked trends. Second, the study may have been too brief to detect trends. Seasons are quite variable, and more than 2 years of sampling may be required to detect seasonal trends. Multiseason (as opposed to seasonal) trends in *X. americanum* (sensu stricto) population levels were detected in Michigan vineyards over a 7-year period (1). Finally, regular seasonal fluctuations in population composition may not be characteristic of certain *Xiphinema* spp. Flegg (7) studied population fluctuation and composition of *X. vuittenezi*, an unidentified *Xiphinema* sp. (similar to *X. americanum*), and *X. diversicaudatum* in England. For all three species, reproductive periods were limited to late spring and summer, and eggs in soil were limited to summer. Regular cycles in levels of adult or juvenile stages were not obvious. Flegg stated that the absence of regular seasonal changes in population density and life stages was due in part to the variable and often lengthy developmental period required for each life stage, the long life span of adults, and the relatively low reproductive rate. In the present study, the predominance of females also indicates that adult *X. americanum* and *X. rivesi* may have long life spans. The putative longevity of adults, the relatively low reproductive rates, the association of these species with stable habitats, and the absence of a pyramid population structure (with many juveniles and few adults) suggest that the life strategies of *X. americanum* and *X. rivesi* are K-selected as opposed to r-selected as discussed by Pianka (21).

Previous studies on *Xiphinema americanum* (sensu lato) in the United States generated few consistent statements regarding seasonal population fluctuation or population composition. In fact, observations on time of population peaks, reproductive periods, and overwintering stages were sometimes contradictory. On alfalfa in Iowa (19), populations peaked in early spring and late summer, gravid females occurred from late May to August, and few adults or juveniles overwintered in frozen soil. On ornamental spruce in Colorado (11), populations peaked from April to August and again from September to January, gravid females occurred in two distinct cycles per year, and eggs, J3, and J4 were the only

overwintering stages. On burley tobacco and an associated fescue–orchardgrass–ladino–red clover sod rotation in Kentucky (8), populations peaked in spring and fall. On cottonwood in South Dakota (18), populations peaked in early summer and early fall and were lowest in April, gravid females were noted from May to July, eggs in soil were observed throughout the year, and all stages except first-stage juveniles overwintered. On grapes in California (6), population levels were higher in fall and winter than in spring and summer. On lilac in Iowa (23), population levels were highest in August, unless the soil moisture was less than 30% field capacity, and gravid females were observed in June and August. *Xiphinema americanum* (sensu lato) is sensitive to soil moisture, soil temperature, and other edaphic factors (10,17,20,22–24); differences in population fluctuation and composition among sites, seasons, and years may therefore be partially explained by differences in edaphic factors.

We first attempted to determine extraction efficiency of the sieving–Baermann funnel method by measuring the relative extraction efficiencies of sieving–Baermann funnel vs. sieving–centrifugation (based on data from Fig. 10) and then directly determining the extraction efficiency of sieving–centrifugation. Results made estimation of extraction efficiency by this method questionable. First, the efficiency of sieving–centrifugation was 15% for nematodes originally extracted by funnel vs. 30% for nematodes originally extracted by centrifuge (Table 3). Second, the relative efficiency of sieving–Baermann funnel and sieving–centrifugation varied with time of year (Fig. 10): sieving–Baermann funnel yielded higher numbers in fall and spring and equivalent numbers in summer. Over the course of this study, the funnel (with nematodes collected after 3 days) yielded about twice as many nematodes as the centrifuge, so we estimated that extraction efficiency by sieving–Baermann funnel was 30–60% (2 × 15% to 30%). Note that similar numbers of nematodes were extracted by centrifugation or funnel in the first extraction test (Table 3); these nematodes were collected from the funnel after 1 day rather than 3 days.

Direct determination of extraction efficiency of sieving–Baermann funnel by addition of known numbers of *Xiphinema* spp. seemed unwise because the sieving–Baermann funnel technique depends on nematode energy, and nematode energy is presumably depleted in obtaining known numbers of nematodes. When this method was tried, however, extraction efficiency was about 66%, and this estimate is probably more reliable than the previous estimate of 30–60%.

The seasonal difference in relative extraction efficiency of the two extraction methods may be due to unrecognized changes in soil or laboratory conditions that differentially affected the extraction methods, or to seasonal changes in nematode activity levels, which would affect recovery of nematodes by the Baermann funnel more than by the centrifugation method. Changes in nematode activity levels might also explain some unusual trends observed in this study. In fall 1984, for example, *Xiphinema* population levels in PA-apple appeared to increase dramatically. The increase could not be due to egg hatch, since an increase in the proportion of J1 was not observed. In fact, the proportions of different stages did not change appreciably during this period. Migration of nematodes from lower to higher depths is unlikely because all stages would not be expected to migrate at similar rates. Sampling error may account for the observed increase in population levels, but an increase in nematode activity might also explain the data. Similarly, the low population levels observed in NY-apple in fall 1984 and the higher levels observed in late winter 1985 may be explained by sampling error or changes in nematode activity.

Division of *X. americanum* (sensu lato) into many different species has not been universally accepted (12,16). The occurrence of *X. americanum* and *X. rivesi* in the same orchard allowed us to study these species under the same macroenvironmental con-

ditions. The similarity in microenvironments occupied by the two species is unknown; however, we frequently have found both *X. americanum* and *X. rivesi* when single cores were extracted (B. A. Jaffee, unpubl.). In the Pennsylvania apple orchard, we easily distinguished two groups of nematodes corresponding to *X. americanum* and *X. rivesi*; however, no striking differences in population fluctuation or composition were noted.

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