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Two New Species of Aphelenchoididae (Nemata) from Declining Red Pines in Maryland[1]

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Abstract: *Aphelenchoides resinosi* n. sp. and *Ektaphelenchus joyceae* n. sp. are described and illustrated from red pines of the Allegheny plateau of Maryland, USA. The new species were found in trees infested with *Bursaphelenchus xylophilus*. Primary diagnostic characters of *A. resinosi* females are constriction of the head, basal stylet knobs, long postuterine sac, two incisures in the lateral field, and conical tail four to five anal body widths long with a simple terminal mucro. Diagnostic characters of the males are two pairs of subventral caudal papillae and spicule shape: Primary diagnostic characters of *E. joyceae* females are a slight constriction of the head, six similar lips, conical tail, and short postuterine sac. Diagnostic characters of the males are spicule size and shape, a single row of spermatocytes, and one pair of caudal papillae. Within-tree distributions of *A. resinosi* and *E. joyceae* are presented. A total of 70% of both red-needled and chlorotic-needled trees in the study were positive for *A. resinosi* and *E. joyceae*. Branch hierarchy was related to the percentage of samples positive for *A. resinosi*.

Key words: *Aphelenchoides resinosi, Bursaphelenchus xylophilus, Ektaphelenchus joyceae,* Maryland, nematode, new species, *Pinus resinosa,* red pine.

Aphelenchoides species have been described from conifers in the United States. Massey (19,20,21) described *A. rhytium* from eastern white pine (*Pinus strobus* L.), loblolly pine (*P. taeda* L.), and red pine (*P. resinosa* Ait.) in New York; *A. conophthori* from eastern white pine in Connecticut; and *A. hylurgi* from eastern white pine in Maine. *Aphelenchoides pityokteini* (21) was described from corkbark fir (*Abies lasiocarpa* var. *arizonica* (Merriam) Lemm.) in New Mexico and *A. polygraphi* (21) from Engelmann spruce (*Picea engelmanni* Parry) in Arizona. Massey (19) found an *Aphelenchoides* sp. of the *A. parietinus* group associated with white fir (*A. concolor* Gord. & Glend. Lindl.) in New Mexico. In studies of pine wilt disease, two populations of an *Aphelenchoides* sp. were found occurring with *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934) Nickle, 1970 in dying pines (9,10,13). One population was found in association with Scots pine (*P. sylvestris* L.) from Missouri (13) and the other in red pine from Maryland (9,10). To date, these populations have remained unidentified.

Another nematode, *Ektaphelenchus* sp., was collected from the same red pines. Massey (17–21) described eight *Ektaphelenchus* species associated with conifers in the United States, three of which have been synonymized with previous taxa (1). *Ektaphelenchus josephi* and *E. riograndensis* were described from limber pine (*P. flexilis* James) and red pine, respectively. *Ektaphelenchus obtusus* was described from Engelmann spruce, *E. prolobos* from corkbark fir, and *E. sandiaensis* from white fir.

The purpose of this paper is to describe two previously undescribed taxa and to provide information on their distribution within dying red pines.

MATERIALS AND METHODS

During the summer of 1987, dying red pines infested with *Bursaphelenchus xylophilus* were collected from Savage River State Forest in Allegany County, Maryland. Trees in that stand were 20–26 years old. Although several stages of decadence were noted among the afflicted trees in red pine forests of Maryland (9,10), only two were chosen for collection: red-needled trees and chlorotic-needled trees. Red-needled trees had uniformly red nee-

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dles and had died in the fall or winter prior to the summer of collection. Chlorotic-needled trees were in early stages of dying during midsummer of the year of collection. Ten red-needled trees were collected in May and June, and ten chlorotic-needled trees were collected in July.

Samples from trunks and branches were taken by means of the method of Harman (9,10). The lowest stem sample was removed approximately 20 cm from the ground, and subsequent samples were taken at 1.5-m intervals. Terminal portions of stems were also collected. Branch hierarchy (primary, secondary, tertiary) and branch decadence (three levels) were utilized in sampling. One branch from each category on each 1.5-m trunk segment was sampled. Samples were collected from the branches at 1.5-m intervals starting at the base of the branch and proceeding outward. Branch samples consisted of cylinders about 10 cm in length.

Nematodes were extracted by placing small samples of wood in 1-oz plastic cups and soaking in tap water for 24 hours. Specimens were heat-killed and preserved in glycerine-formaldehyde. Temporary mounts were made in lactophenol cotton blue after gently heating the nematodes in a cavity slide containing a drop of tap water.

Nematodes extracted from each sample were counted. Final nematode counts were expressed as number of nematodes per gram of dry wood. Sample dry weight was determined following the method of Brown and Bethel (4). Samples were oven-dried at 100 C after nematode extraction and the weights recorded over a 48-hour period. Drying was stopped when weights remained constant over time, and the constant weight was recorded as the dry weight of the sample. Eighty-three stem samples and 101 branch samples were analyzed from red-needled trees, and 77 stem samples and 97 branch samples from chlorotic-needled trees.

Permanent mounts of nematodes were made in anhydrous glycerine after processing them slowly to glycerine by Hooper's method (11). Drawings were done with a microscope fitted with a drawing attachment. All measurements are in micrometers (μm) unless otherwise specified.

Systematics

Aphelenchoides resinosi n. sp. (Fig. 1A-E)

Measurements

Holotype (female in glycerine): L = 506; stylet = 12; a = 27; b = 9; c = 15; c′ = 2.4; V = 72.

Female (n = 40): L = 400–800 (mean = 530; SD = 110); stylet = 10–11 (mean = 10.6; SD = 0.6); ratios-a = 29–53 (mean = 35; SD = 5), b = 7–13 (mean = 9; SD = 2), c = 12–19 (mean = 15; SD = 2), c′ = 3–4 (mean = 3.4; SD = 0.4), V = 66–79 (mean = 69; SD = 2.8).

Allotype (male in glycerine): L = 484; stylet = 11; spicule = 15; a = 26; b = 7; c = 16; c′ = 2.3.

Male (n = 26): L = 360–480 (mean = 450; SD = 60); stylet = 10 (mean = 10; SD = 0); spicule = 13–15 (mean = 14; SD = 0.6); ratios-a = 31–48 (mean = 36; SD = 4), b = 6–10 (mean = 8; SD = 1), c = 11–19 (mean = 14; SD = 2), c′ = 2–3 (mean = 2.4; SD = 0.1).

Description

Female: Body slender, slightly curved ventrally upon relaxation with heat. Cuticle with fine, transverse striae. Lateral field with two incisures. Lip region and tail terminus not annulated. Body tapering posteriorly to conoid terminus. Terminus with small, fine mucro. Head set off by constriction (Fig. 1C); lips with guiding rods. Stylet delicate, basal knobs small. Stylet narrowed anteriorly to fine tip. Tip of stylet very faint in preserved and fresh specimens. Median bulb spherical with well-developed, centrally situated valves. Duct pores into median bulb sometimes visible. Nerve ring 3/4 of body width posterior from base of median bulb, encircling esophageal gland lobes and anterior end of intestine. Esophageal gland lobes three body widths long. Excretory pore at level

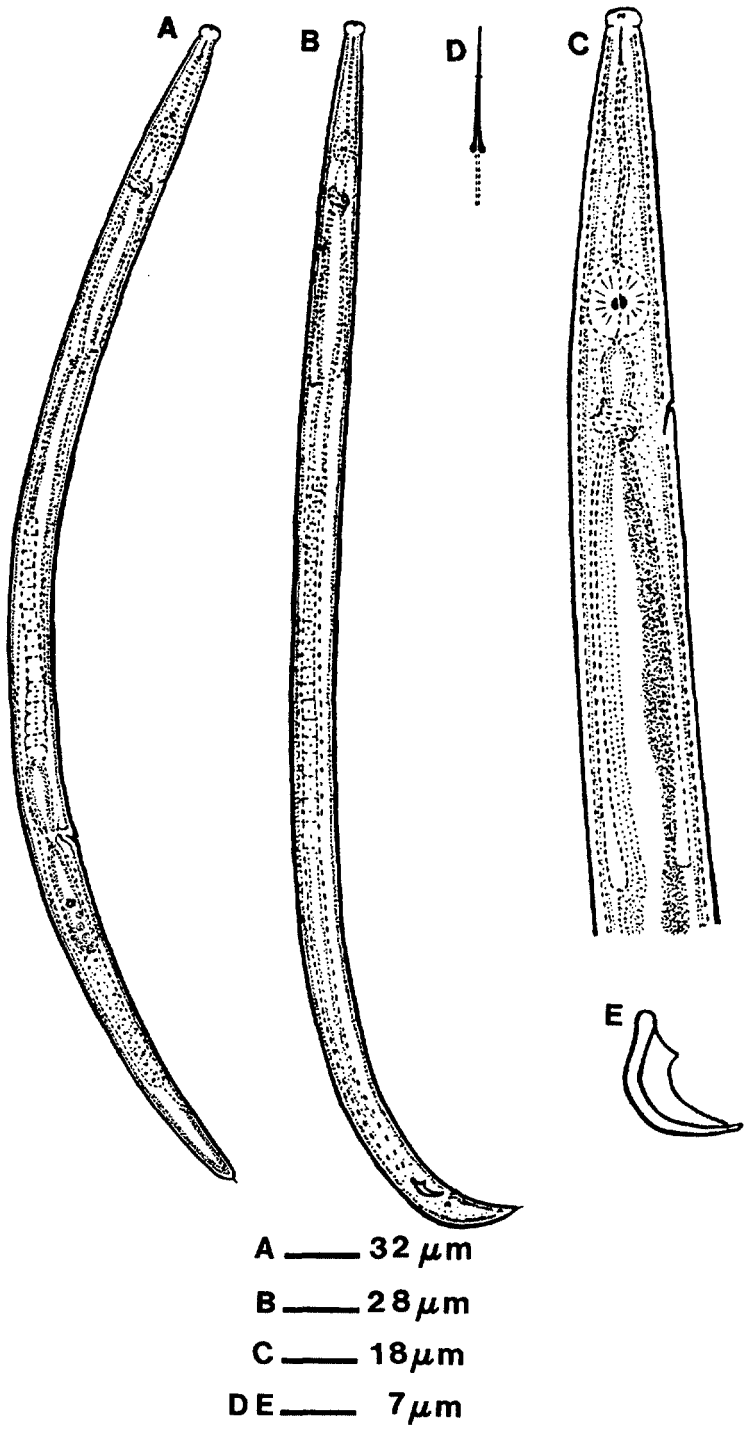

FIG. 1. *Aphelenchoides resinosi* n. sp. A) Full view of female. B) Full view of male. C) Head and neck of female. D) Stylet. E) Spicule.

of nerve ring. Vulva slightly protuberant. Vagina narrow, oblique. Ovary outstretched, not overlapping esophageal gland lobes. Oocytes in single row. Gonad length 115 μm. Postuterine sac 49 μm long, sometimes collapsed.

Male: Male shorter than female, body J-shaped. Cuticle finely annulated, lip region and tail terminus smooth. Head set off, lips bearing guiding rods. Excretory pore, median bulb, and nerve ring same as female. Testis long, outstretched. Tail tapering to conoid terminus with small, sharp mucro. Bursa and gubernaculum absent. Two pairs of subventral caudal papillae present: first pair adanal, second post-anal, about one body width anterior to tail tip. Spicules paired, typically aphelenchoid, arcuate and rose-thorn shaped; condylus rounded and prominent, rostrum pointed and less prominent. Point of intersection of dorsal and ventral limbs not visible.

Type host and locality

From the xylem of stems and branches of 20–26-year-old dying red pines (*Pinus resinosa* Ait.) infested with *Bursaphelenchus xylophilus,* Savage River State Forest, Compartment 42, Allegany County, Maryland, USA.

Type specimens

Holotype (female): Collected 6 June 1987, by T.R. Kaisa, A.L. Harman, and D.M. Harman in Allegany County, Maryland, USA. Slide no. T-513t, deposited in the U.S. Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland. *Allotype (male):* Same data as holotype. Slide no. T-514t, deposited in USDANC, Beltsville, Maryland. *Paratypes (7 females and 5 males):* Same data as holotype, deposited in USDANC, Beltsville, Maryland; Nematology Department, Rothamsted Experimental Station, Harpenden, Herts., England; and Frostburg State University, Department of Biology, Frostburg, Maryland.

Diagnosis

The female of *A. resinosi* averages 530 µm in length with a slender body, annulated cuticle, and two incisures in the lateral field. The tail is conoid with a simple mucro. The stylet is short (11 µm) with basal knobs. The male of *A. resinosi* averages 450 µm in length with a conoid, mucronate tail bearing two pairs of subventral caudal papillae. Spicules are 14 µm long, arcuate with a prominent condylus.

Relationships

In the female body length of 400–800 µm, the presence of two incisures in the lateral field, and the presence of a simple mucro, *A. resinosi* is similar to *A. arachidis* Bos, 1977 (3), *A. fragariae* (Ritzema-Bos, 1891) Christie, 1932 (5), and *A. helicosoma* Maslen, 1979 (16). It differs from *A. arachidis* in the shorter male body (360–480 µm vs. 560–1040 µm) and in the number of caudal papillae (two vs. three pairs in *A. arachidis*). *Aphelenchoides fragariae* is distinguished from *A. resinosi* by the longer male body (480–650 µm) and the presence of three pairs of caudal papillae. *Aphelenchoides helicosoma* differs in the stouter female body (a = 25–32 vs. 29–53), stouter male body (a = 26–37 vs. 31–48), presence of a stylet guiding ring, longer male body (505–925 µm), and in the presence of three pairs of caudal papillae.

Aphelenchoides resinosi is similar to *A. conimucronatus* Bessarabova, 1966 (2) and *A. vigor* Thorne & Malek, 1968 (26) in having two incisures and a simple mucro. *Aphelenchoides conimucronatus* differs in the shorter female body of 280–440 µm, shorter spicule of 10 µm vs. 13–15 µm, and spicule shape. The spicules of *A. conimucronatus* are smoothly curved with a non-prominent condylus, whereas those of *A. resinosi* have a distinct condylus and irregular curvature. *Aphelenchoides vigor* differs in the longer female body of 900 µm, longer male body of 720 µm, smaller V of 63 vs. 66–75 for *A. resinosi,* the presence of three pairs of caudal papillae, and a bluntly rounded spicule terminus versus a pointed terminus in *A. resinosi.*

In the female body length of 410–550 µm, male body length of 380 µm, stylet length of 10–12 µm, and in the presence of two pairs of caudal papillae, *A. bicaudatus* (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941 (8) is similar to *A. resinosi.* It can be distinguished from *A. resinosi* by

the bifurcation of the female tail, and in the presence of a stylet guiding ring.

Aphelenchoides appendurus Singh, 1967 (25) is similar to *A. resinosi* in having two incisures and V of 66–69.8, but differs from it in the longer female body of 720–880 μm, longer male body of 810 μm, longer stylet of 16.5–17.5 μm vs. 10–11 μm, and in the presence of a leaf-like mucro.

Aphelenchoides asterocaudatus Das, 1960 (7) is similar to *A. resinosi* in the female body length of 620 μm, in having two incisures, and V of 68. It differs from *A. resinosi* in the presence of a star-shaped mucro. Males are unknown for *A. asterocaudatus*.

Aphelenchoides rosei Dmitrenko, 1966 (6) is similar to *A. resinosi* in having two incisures, but differs in the longer male stylet of 14 μm, longer male body of 670 μm, and a nonmucronate male tail. Females are unknown for *A. rosei*.

Aphelenchoides brevistylus Jain & Singh, 1984 (12) and *A. unisexus* Jain & Singh, 1984 (12) are similar to *A. resinosi* in having two incisures; in the female body lengths of 390–630 μm and 480–760 μm, respectively; and in V of 63–71 and 62.3–67.2, respectively. *Aphelenchoides brevistylus* males are similar to *A. resinosi* males in spicule length of 14–16 μm and in having two pairs of caudal papillae. Males are unknown for *A. unisexus*. Both *A. brevistylus* and *A. unisexus* can be distinguished from *A. resinosi* by their star-shaped mucros. *Aphelenchoides brevistylus* also differs in having a shorter stylet of 6–8 μm and in the absence of stylet knobs.

Ektaphelenchus joyceae n. sp.
(Fig. 2A-G)

Measurements

Holotype (female in glycerine): L = 710; stylet = 17; a = 36; b = 9; V = 83.

Female (n = 32): L = 550–740 (mean = 640; SD = 60); stylet = 14–18 (mean = 16; SD = 1); ratios- a = 29–44 (mean = 34; SD = 3), b = 7–13 (mean = 9; SD = 1), V = 77–86 (mean = 82; SD = 2).

Male (n = 7): L = 400–470 (mean = 440; SD = 30); stylet = 14–16 (mean = 15; SD = 0.8); spicule = 13–14 (mean = 14; SD = 0.3); ratios- a = 26–35 (mean = 32; SD = 3), b = 5–7 (mean = 6; SD = 0.6), c = 15–18 (mean = 16; SD = 1), c′ = 1.8–2.2 (mean = 2; SD = 0.2).

Description

Female: Body ventrally arcuate, tapering to conoid tail. Cuticle with fine, transverse striae. Head slightly set off by slight constriction. Tail terminus with lateral spike in 31% of females. Stylet delicate, without knobs. Stylet tip opening slanted ventrally (Fig. 2C). Median bulb oblong, longer than wide, one body width long; valves well developed, post-median. Excretory pore one-half body width below nerve ring; hemizonid just anterior to excretory pore. Nerve ring one body width posterior to median bulb. Esophageal gland lobes long, about five times body width, overlapping intestine dorsally. Vulva not protuberant, flap absent. Vagina short, narrow, oblique. Rectum and anus absent; intestine terminating in blind sac. Ovary outstretched. Oocytes in two rows. Spermatheca oblong, sperm visible in 47% of females. Postuterine sac short, length about one-half corresponding body width.

Male: Male shorter than female. Body J-shaped or curved ventrally when relaxed. Tail curved sharply through 180°, tail region narrowing to pointed tip. Testis outstretched, spermatocytes in single row. Spicules arcuate, condylus rounded, prominent; rostrum small, pointed. One pair of ventral caudal papillae near tail tip. Bursa and gubernaculum absent.

Type host and locality

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Type specimens

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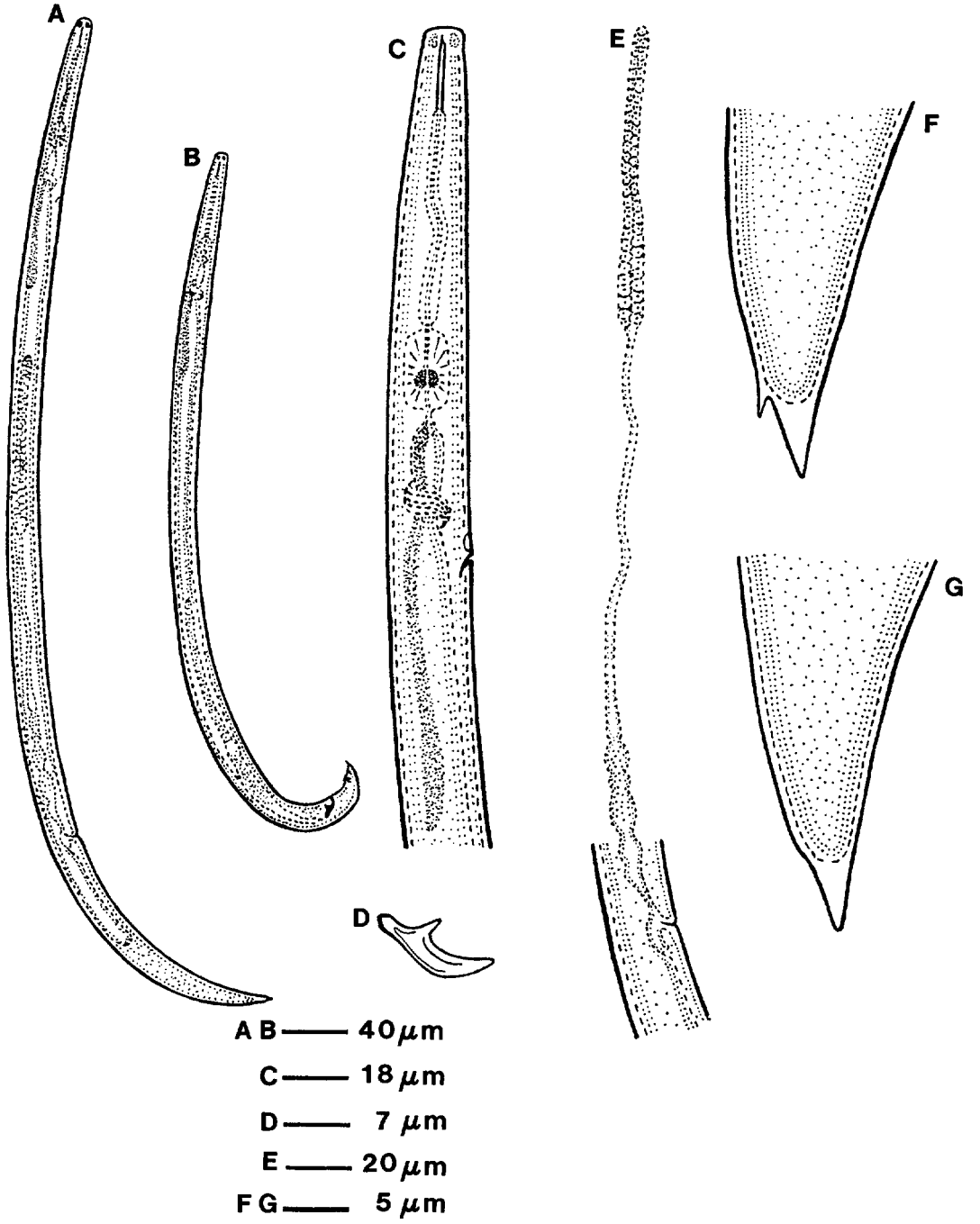

FIG. 2. *Ektaphelenchus joyceae* n. sp. A) Full view of female. B) Full view of male. C) Head and neck of female. D) Spicule. E) Female gonad. F) Female tail terminus with lateral spike. G) Simple female tail terminus.

Harman in Allegany County, Maryland, USA. Slide no. T-515t, deposited in the U.S. Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland. *Paratypes (8 females and 3 males):* Same data as holotype, deposited in USDANC, Beltsville, Maryland; Nematology Department, Rothamsted Experimental

Station, Harpenden, Herts., England; and Frostburg State University, Department of Biology, Frostburg, Maryland.

Diagnosis

The female of *E. joyceae* averages 640 μm long with a moderately set-off head. The tail terminus is conoid at times with a small lateral spike. The stylet is 16 μm long and without basal knobs. Oocytes are in two rows, the vagina is oblique, and the postuterine sac is less than one body width long. The males of *E. joyceae* are short (440 μm) with pointed, conoid tails bearing one pair of ventral caudal papillae. Spermatocytes are in a single row, and spicules are small (14 μm) and smoothly curved with a prominent condylus and a small, pointed rostrum.

Relationships

In the female body length of 550–740 μm, stylet length of 14–18 μm, and in having two rows of oocytes, *E. joyceae* is similar to *E. larici* Lazarevskaya, 1963 (15), *E. macrobulbosus* Rühm, 1956 (24), *E. prolobos* Massey 1964 (18), *E. sandiaensis* Massey, 1964 (19), and *E. tuerkorum* Rühm, 1956 (24). It differs from *E. larici* in the short postuterine sac (less than one body width vs. six body widths long) and in the absence of guiding rods in the lips. Males of *E. larici* are unknown. *Ektaphelenchus joyceae* differs from *E. macrobulbosus* in the arrangement of spermatocytes (one vs. two rows), number of caudal papillae (one vs. two pairs), and in the rounded versus hooked spicule terminus of *E. macrobulbosus*. *Ektaphelenchus joyceae* differs from *E. prolobos* in having a stylet without knobs, in the shorter male body of 400–470 μm vs. 610–660 μm, in the presence of one vs. two rows of spermatocytes, and in the number of caudal papillae (one vs. two pairs). *Ektaphelenchus sandiaensis* differs in having a longer stylet of 19.5–22 μm vs. 14–18 μm, in the bluntly rounded female tail terminus vs. conical, and in having a postuterine sac that is twice as long as that of *E. joyceae*. *Ektaphelenchus sandiaensis* males differ from *E. joyceae* males in the longer stylet of 21 μm and in the presence of two pairs of caudal papillae. A longer male body of 645–690 μm and two pairs of caudal papillae distinguish *E. tuerkorum* from *E. joyceae*.

With a stylet length of 16 μm, *E. amitini* (Fuchs, 1937) Rühm, 1956 (24) is similar to *E. joyceae*, but differs by having stylet knobs and a shorter body of 523 μm vs. 550–740 μm in *E. joyceae*. Males are unknown for *E. amitini*.

Ektaphelenchus goffarti Rühm, 1956 (24) is similar to *E. joyceae* in the female body length of 547–701 μm and male body length of 442–485 μm. It differs from *E. joyceae* in having one row of oocytes vs. two, in the presence of two rows of spermatocytes vs. one, and in having three pairs of caudal papillae vs. one in *E. joyceae*.

In the conical female tail and stylet without knobs, *E. joyceae* is similar to *E. betulae* Rühm, 1956 (24), *E. obtusus* Massey, 1956 (17), *E. riograndensis* Massey, 1964 (18), *E. scolyti* Rühm, 1956 (24), *E. skrjabini* Lazarevskaya, 1962 (14), and *E. zwölferi* Rühm, 1956 (24). *Ektaphelenchus betulae, E. obtusus, E. riograndensis, E. scoltyi,* and *E. zwölferi* all differ from *E. joyceae* in having longer female bodies of greater than 740 μm, longer male bodies of greater than 600 μm, and in the presence of three pairs of caudal papillae. *Ektaphelenchus skrjabini* differs in the shorter female body of 466–508 μm and in having three pairs of caudal papillae.

Ektaphelenchus josephi Massey, 1974 (21) is similar to *E. joyceae* in the conical female tail, in having two rows of oocytes, and a short postuterine sac (less than one body width long). It differs from *E. joyceae* in the presence of stylet knobs, longer female body of 850–950 μm, longer male body of 670 μm, and in having two pairs of caudal papillae and two rows of spermatocytes.

Discussion

The bionomics of *Aphelenchoides resinosi* and its mode of transmission to red pines

TABLE 1. Occurrence of *Aphelenchoides resinosi*, n. sp. (+) and its co-occurrence with *Bursaphelenchus xylophilus* (B) and *Ektaphelenchus joyceae* n. sp. (E) by sample category in Maryland red pines.

Red-needled trees				Chlorotic-needled trees			
Tree number	Trunk	Primary branches	Secondary branches	Tree number	Trunk	Primary branches	Secondary branches
1	–	–	–	11	–	+	–
2	–	–	–	12	–	–	+,B
3	–	–	+	13	–	+	+
4	–	–	–	14	–	–	–
5	–	+	+,B	15	–	+	+
6	+,E	+	+	16	+	+	–
7	–	+,E	+	17	+	+	+
8	–	+,B	+	18	–	–	–
9	+,B	+	+	19	–	–	+
10	–	+	–	20	+	+,B	+

are unknown. Possibly, it is a mycophagous species that is carried by insects associated with pines, as Massey (21), Nickle (22), and Nickle and Hooper (23) have reported associations of aphelenchs with pine bark beetles.

Distributions of *A. resinosi, E. joyceae,* and *B. xylophilus* were determined and compared (Table 1). *Aphelenchoides resinosi* co-occurred with *B. xylophilus* in both trunk and branch samples from red-needled trees, but only in branch samples from chlorotic-needled trees. *Aphelenchoides resinosi* co-occurred with *E. joyceae* only in red-needled trees.

Seventy percent of red-needled trees contained *A. resinosi.* Occurrence was more frequent in branch than in trunk samples (Table 1). Eighty percent of chlorotic-needled trees contained *A. resinosi* and had similar frequency of occurrence in primary and secondary branch samples. Average *A. resinosi* per-gram sample weight was higher among secondary branches than primary branches (Table 2). In both types of trees, average nematodes per sample weight from primary branch samples were about one-third the number from secondary branch samples. Branch hierarchy was related to percentages of samples containing *A. resinosi.* The number of samples containing the nematode was greater in secondary branches than in primary branches ($P = 0.002$). *Ektaphelenchus joyceae* was sparsely distributed in the sampled trees, occurring in two of 10 red-needled trees and in none of the chlorotic-needled trees.

The respective roles of *A. resinosi* and *E. joyceae* in the bioecology of dying red pines was not determined in this study. Their frequencies were relatively low in all sample categories compared to that of *B. xylophilus.* Massey (21) stated that aphelenchoid nematodes were often associated with bark beetles phoretically, and it seems likely that *A. resinosi* and *E. joyceae* are also insect associates, not tree pathogens.

TABLE 2. Occurrence of *Aphelenchoides resinosi* n. sp. by numbers in red-needled and chlorotic-needled red pine branches.

	Total dry weight of samples (g)		Total number of nematodes		Average number of nematodes/g	
	Primary branches	Secondary branches	Primary branches	Secondary branches	Primary branches	Secondary branches
Red-needled trees	189	5	821	63	4	13
Chlorotic-needled trees	63	3	44	68	7	22

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