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Morphometrics, Illustration, and Histopathology of *Sphaeronema rumicis* on Cottonwood in Utah

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Abstract: The morphology of a population of *Sphaeronema rumicis* Kir'yanova found on cottonwood in Utah is illustrated by light and scanning electron micrographs, as well as by drawings. This is the first report of males of *S. rumicis*, a species also not known previously to occur in North America. *S. rumicis* females on cottonwood in the United States were smaller than those found by Kir'yanova on sorrel in the USSR. Females and second-stage juveniles (J2) from the United States had slightly shorter stylets than did females and J2 from the USSR. Males were vermiform and had degenerate esophagi. On secondary cottonwood roots *S. rumicis* induces formation of a syncytium originating from proliferated pericyclic cells. Thick outer walls, wall protuberances, absence of cell wall ingrowths, dense cytoplasm, and hypertrophied nuclei were the main characteristics of syncytia observed in *S. rumicis*-infected cottonwood roots.

Key words: cottonwood, histopathology, host response, morphology, parasitic habit, *Populus angustifolia*, scanning electron microscope, semiendoparasitic nematode, *Sphaeronema rumicis*, syncytium.

In 1970 Kir'yanova (6) described *Sphaeronema rumicis* from sorrel (*Rumex confertus* Willd.) in the USSR. The nematode was found for the first time in North America near Salt Lake City, Utah, infesting narrowleaf cottonwood (*Populus angustifolia* James) in a forest in the Little Cottonwood Canyon at an elevation of about 1,500 m. Kir'yanova did not find males, but males were found at the Utah site. Of the members of the Tylenchulidae, only the morphology of *Trophotylenchulus floridensis* Raski, *Tylenchulus furcus* Van Den Berg and Spaull, and *T. semipenetrans* Cobb has been illustrated by scanning electron microscopy (SEM) (3,12). Little information exists on the pathogenicity of *Sphaeronema* species.

The objectives of this study were to 1) illustrate the morphology of *S. rumicis* by light microscopy and SEM, 2) compare the morphometrics of the Utah and Russian populations, 3) describe the male of the species, and 4) study the histopathology of *S. rumicis* in cottonwood roots.

MATERIALS AND METHODS

Males and second-stage juveniles (J2) of *S. rumicis* were extracted from soil by Cobb's sieving and decanting method (2); females were collected directly from cottonwood roots. Males, J2, and females for morphometric data and drawing were killed and fixed in hot aqueous 4% formaldehyde + 1% propionic acid, dehydrated in ethanol vapor, and mounted in dehydrated glycerin (4). Eggs were fixed and mounted in 2.5% formalin.

Females and J2 for SEM were killed and fixed in 4:1 formalin-propionic acid, transferred to 1% osmium tetroxide solution for 12 hours, infiltrated with Spurr's resin, and mounted on SEM stubs (1). Specimens were coated with gold and observed in the SEM at 5 kV accelerating voltage.

Cottonwood roots infected with *R. rumicis* were collected in December. Roots were gently washed free of soil, cut into 4–5 mm lengths, fixed in FAA, dehydrated in a tertiary butyl alcohol series, and embedded in paraffin. Embedded roots were sectioned at 10–15 μm; sections were stained with safranin and fast-green, mounted in Dammar xylene, and examined under a compound microscope.

RESULTS

Sphaeronema rumicis is a semiendoparasitic nematode. J2 are vermiform; they penetrate cottonwood roots approximately ⅓ the body length with the remaining posterior portion protruding outside the root (Fig. 1A). Adult females are sedentary; the posterior portion of the body swells to become spherical (Fig. 1B). As females approach maturity, they extrude a gelatinous matrix (Fig. 1C) in which the eggs are deposited. We observed about 65

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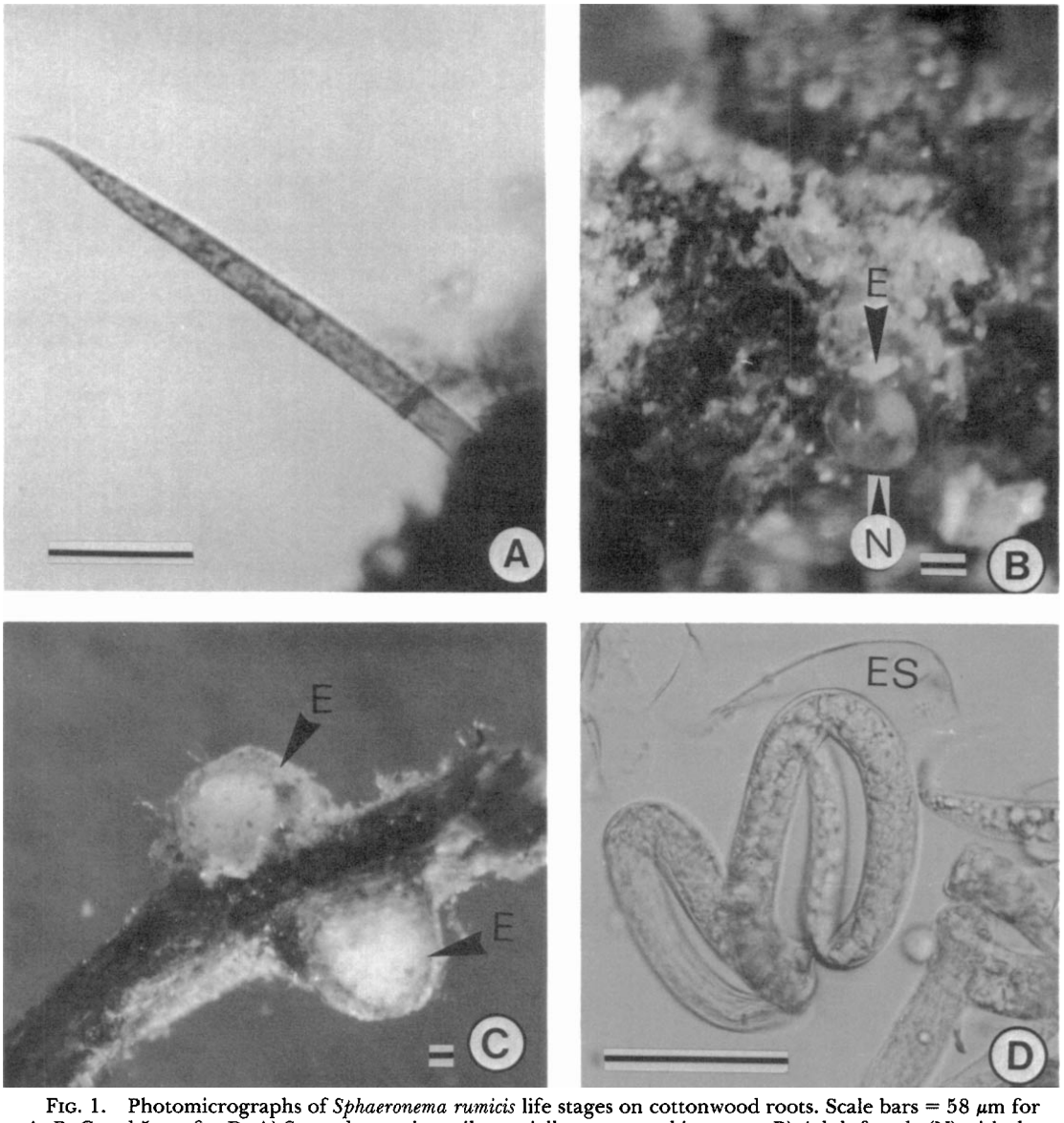

FIG. 1. Photomicrographs of *Sphaeronema rumicis* life stages on cottonwood roots. Scale bars = 58 μm for A, B, C and 5 μm for D. A) Second-stage juvenile partially penetrated in a root. B) Adult female (N) with the enlarged posterior portion protruding from root (gelatinous matrix removed). E = egg. C) Gelatinous matrix (E) filled with eggs covering mature female. D) Coiled second-stage juvenile released from the egg shell (ES).

eggs per egg mass, whereas 80 was the average number reported on sorrel (6).

Morphometrics

Egg (n = 35): S. rumicis eggs on cottonwood were smaller than those found on sorrel by Kir'yanova (6); 92 μm (84–99) × 45 μm (43–47) vs. 104 μm (98–116) × 48 μm (39–53). L/W ratio was 2.1 (1.9–2.4). The egg shell was unsculptured and hyaline, and J1 or J2 were folded five times inside the egg shell (Fig. 1D). Intrauterine

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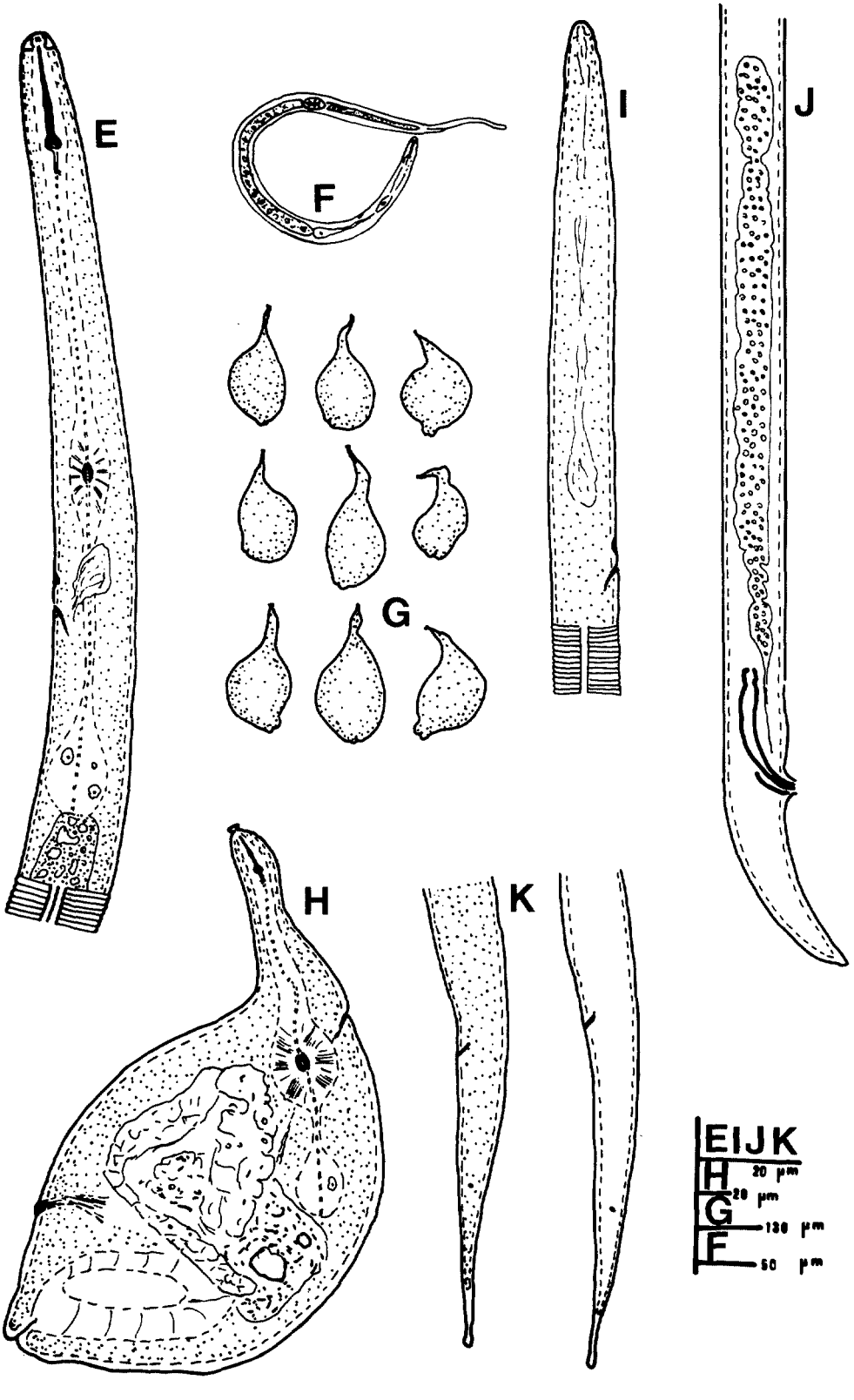
FIG. 2. Drawings of *Sphaeronema rumicis* life stages. E) Body anterior portion of a second-stage juvenile. F) Second-stage juvenile entire body. G) Representative shapes of adult females. H) Adult female. I) Male anterior body. J) Male posterior body. K) Tails of second-stage juveniles.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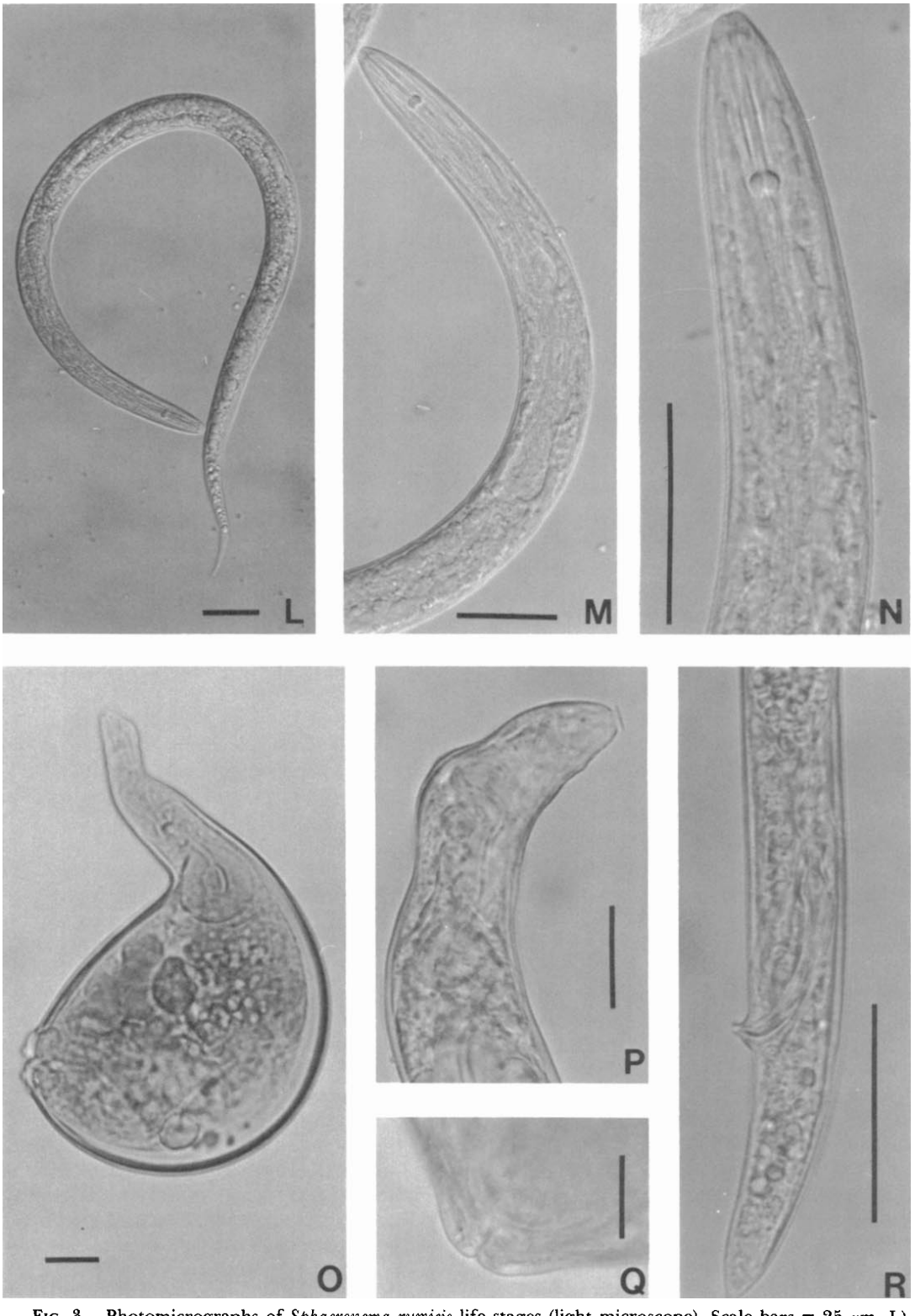

FIG. 3. Photomicrographs of *Sphaeronema rumicis* life stages (light microscope). Scale bars = 25 μm. L) Second-stage juvenile. M) Esophageal region of second-stage juvenile. N) Anterior end of second-stage juvenile. O) Adult female. P, Q) Adult female anterior and posterior end. R) Male posterior region. Note the protruding cuticle at cloacal opening.

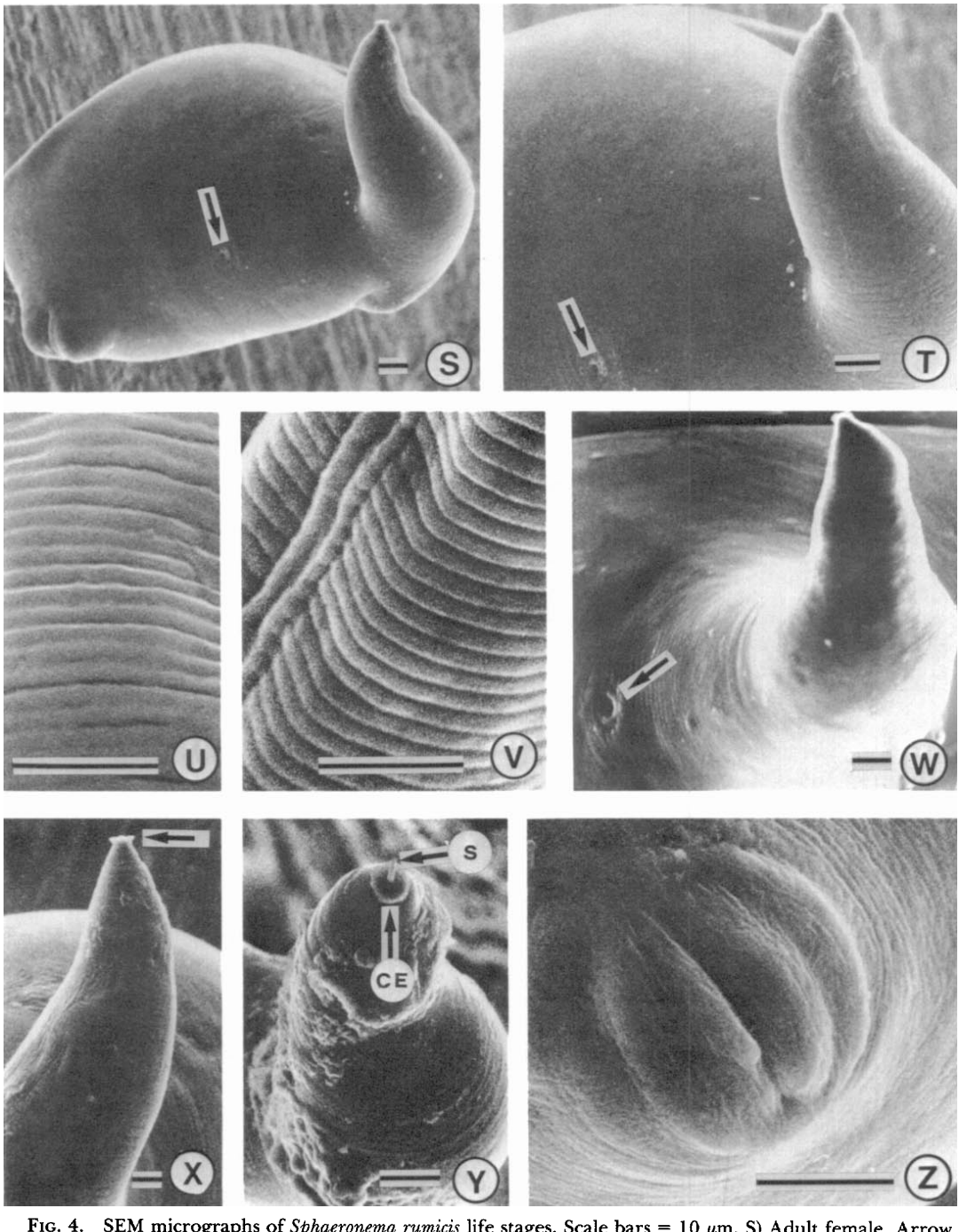

FIG. 4. SEM micrographs of *Sphaeronema rumicis* life stages. Scale bars = 10 μm. S) Adult female. Arrow indicates anus. T) Anterior body portion of an adult female. Arrow indicates anus. U) Adult female cuticle annulation. V) Annulation and lateral field of second-stage juvenile. W) Excretory pore (arrow) at the base of adult female neck. X) Adult female neck showing the discoid first head annule (arrow). Y) Circum-oral elevation (CE) en face view. S = stylet. Z) Vulval region of female, with protruding lips.

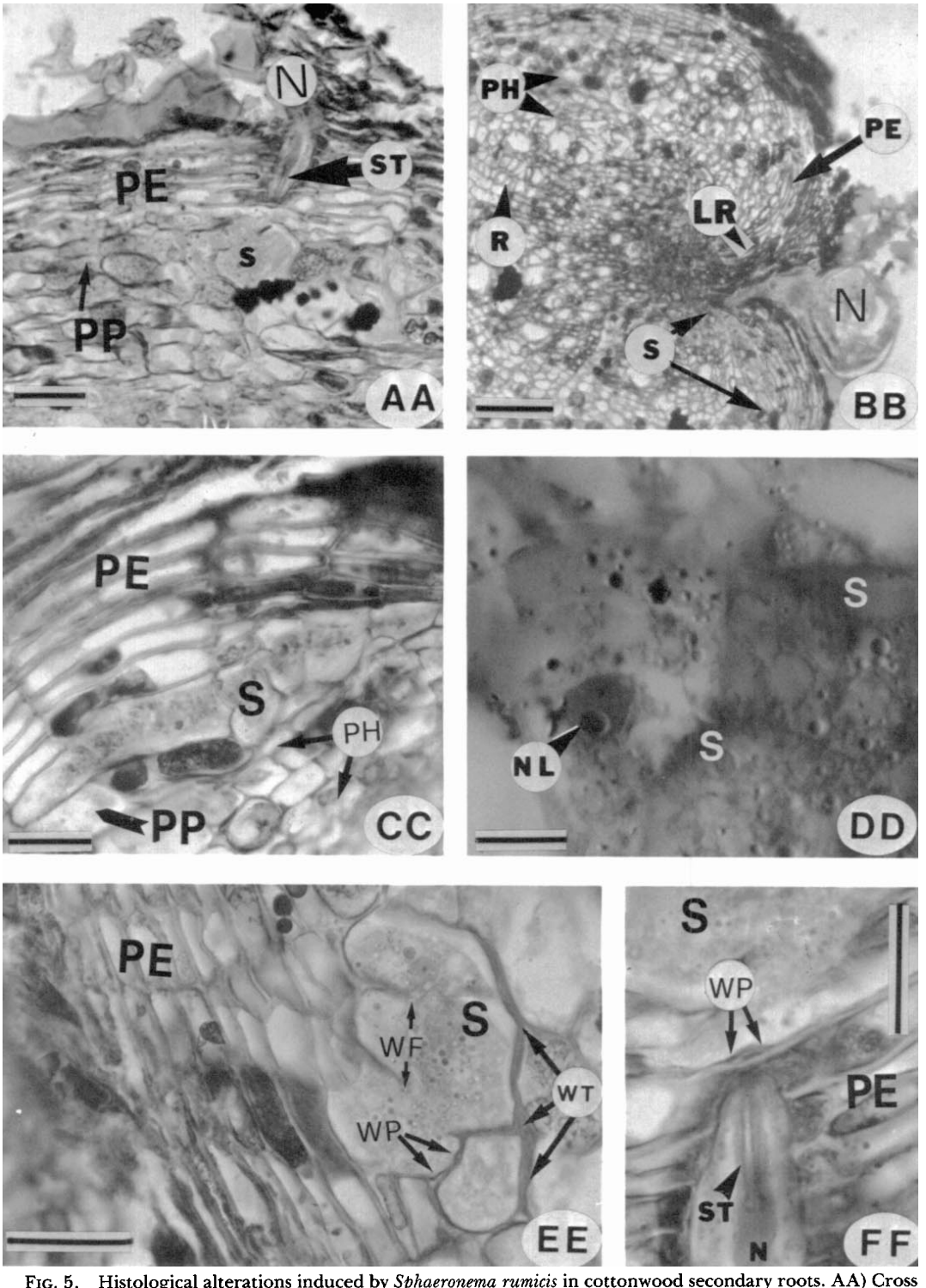

FIG. 5. Histological alterations induced by *Sphaeronema rumicis* in cottonwood secondary roots. AA) Cross section showing a nematode (N) with the anterior body portion penetrated into the periderm (PE) and feeding from a syncytium (S) formed in the proliferated pericycle (PP). ST = stylet. Scale bar = 21 μm. BB) Cross section of a root showing a nematode (N) in invaginated periderm (PE) at a lateral root axil (LR). PH = phloem, R = vascular ray, and S = syncytium. Scale bar = 100 μm. CC) Cross section showing syncytium (S) derived from proliferated pericycle (PP) and incorporating phloem elements (PH). PE = periderm. Scale

eggs averaged from 90 × 43 to 99 × 47 μm.

Second-stage juvenile (n = 40): Body slender, tapering at both ends (Figs. 2E, F, K; 3L, M, N). L = 510 μm (486–523), a = 35 (33–38), b = 3.6 (3.5–3.8), c = 8.7 (8.3–9.0). Length of stylet slightly shorter than that of J2 from sorrel (6): 20 μm (19–21) vs. 21 μm (20–23). Additional morphological details not reported in the original description (6) were 1) cuticular annulations fine but distinct, 1.4 μm wide (Fig. 4V); 2) lateral field, with three equidistant incisures, approximately ¼ as wide as the body width (Fig. 4V); 3) esophagus well developed, extending 130–140 μm from anterior end (Fig. 3M); 4) anus not always easily observed, located 55 μm (53–63) from tail terminus; 5) oval genital primordium situated at 130–135 μm from terminus; and 6) pore-like phasmids 29 μm (26–32) from terminus.

Female (n = 20): Body subspherical similar to that of *Meloidogyne* spp. (Figs. 2G, H; 3O; 4S, T). Neck well defined (Figs. 3P, 4W, X, Y). Vulval protrusion prominent (Figs. 3Q, 4Z). *S. rumicis* females on cottonwood were smaller than those found on sorrel (6), their width being 0.13 mm (0.11–0.15) vs. 0.15 mm (0.14–0.20) and their length 0.24 mm (0.18–0.27) vs. 0.28 mm (0.24–0.34). L/W ratio 1.3 (1.2–1.6). Stylet length slightly shorter than that of females from sorrel (6): 21 μm (20–23) vs. 24 μm (20–27). Cuticle 5 μm (4–6) thick, marked by fine striae 1.4–1.7 μm wide (Fig. 4U). First head annule (6–9 μm) wider than the second (Fig. 3P), appearing as a cup-like circum-oral elevation (Fig. 4X, Y). Metacorpus 39 μm (38–40) in diameter, the same as that of females from sorrel (6). Dorsal esophageal gland orifice (DGO) 5.5 μm (5–6) behind stylet knobs, vs. 8 μm (7–9) in females from sorrel (6). Excretory pore located in a slight depression of the cuticle (Fig. 4W) 102 μm (95–112) from anterior end, vs. 110 μm (81–133) in females from sorrel (6). Anus dorsally situated, 68 μm (63–73) from vulva, vs. 64 μm (35–91) in the Russian population. SEM shows the anus and excretory pore as pore-like apertures in slight depressions of the cuticle (Fig. 4T, W). Vulva slit-like, terminal (Fig. 4Z) surrounded by prominent protruding and unsculptured vuval lips (Figs. 3Q, 4Z).

Male (n = 20): Vermiform, found in the soil with J2 or in egg masses (Figs. 2I, J; 3R). Many egg masses on cottonwood roots collected in December lacked males; no more than one male was detected per egg mass. L = 473 μm (430–500), a = 46 (42–50), c = 13 (12–15), stylet absent. Lip region smooth, not set off from the body. Body cuticle with fine annules 1.2 μm wide; esophagus degenerate. Excretory pore 96–100 μm from anterior end. Cuticle protruded at cloacal opening (Figs. 2J, 3R). Caudal alae absent; lateral field with two longitudinal incisures. Single testis 132 μm (100–150) long, 27% (23–30) of body length (Fig. 2J). Spicules 21–23 μm long, curved ventrally (Figs. 2J, 3R). Gubernaculum simple, 6–7 μm long. Tail conoid with rounded terminus (Fig. 3R).

Histopathology

Only secondary roots of cottonwood collected in December were infected with *S. rumicis.* females in these roots were observed with the anterior portion of the body penetrating into the periderm (Fig. 5AA). In some cases nematodes penetrated invaginated periderm at lateral root axil (Fig. 5BB). *S. rumicis* established a permanent feeding site in the proliferated pericycle cells adjacent to the periderm to form a syncytium (Fig. 5AA, CC). Cells at the feeding site were enlarged with dense cytoplasm (Fig. 5CC). During syncytium expansion, adjacent phloem cells and proliferated pericyclic were incorporated into the syncytium (Fig. 5CC). Syncytial nuclei were usually hypertrophied with prominent nucleoli (Fig. 5DD), averaging 6 μm wide × 8 μm long, whereas normal pericyclic nuclei averaged 3.5 μm wide × 4.5

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bar = 25 μm. DD) Cross section showing a syncytium (S) with hypertrophied nucleus and prominent nucleolus (NL). Scale bar = 8 μm. EE) Cross section showing a mature syncytium (S) with wall fragments (WF), wall protuberances (WP), and thickened outer walls (WT). PE = periderm. Scale bar = 25 μm. FF) Cross section showing a nematode (N) penetrated in the periderm (PE) and feeding from a syncytium (S). Note prominent wall protuberance (WP) at feeding site. ST = stylet. Scale bar = 22 μm.

μm long. There was no evidence of mitotic activity inside syncytia. During cell fusions walls of adjacent cells dissolved, leaving wall fragments inside the syncytium (Fig. 5EE). Outer walls of mature syncytia were irregularly thick adjacent to pericyclic cells and at the nematode feeding site (Fig. 5EE). Syncytial walls were 3.7 μm thick vs. 2.3 μm for normal xylem cells. The syncytium outer walls contained protuberances delimiting depressions that probably were pit fields (Fig. 5EE). A very large wall protuberance, about 3.5 μm thick, was observed at one nematode feeding site (Fig. 5FF). No cell wall ingrowths, as reported in syncytia caused by *Globodera* and *Heterodera* species (5), were observed in syncytia induced by *S. rumicis*. *S. rumicis* syncytia averaged about 31 μm wide × 128 μm long.

Discussion

S. rumicis and *S. californicum* Raski and Sher are distributed in cool regions—*S. rumicis* in Utah and in northeastern USSR and *S. californicum* in El Dorado County, California, at an elevation of about 1,800 m (8). Other reported species were found in tropical and subtropical climates (9,11).

The parasitic habits of *S. rumicis* on cottonwood are similar to those of *Tylenchulus semipenetrans* on citrus (13) in that J2 are the infective stages, both juveniles and females are semiendoparasitic, and males are not parasitic. However, *S. rumicis* induces formation of specialized cells in the host root tissue that are different from those caused by *T. semipenetrans*. Nurse cells with hypertropied nuclei are formed in the root cortex by *T. semipenetrans* (5), whereas with *S. rumicis* a syncytium is induced in proliferated pericyclic cells also involving phloem cells. The anatomical alterations caused by *S. rumicis* differ also from nurse cells caused by *Trophotylenchulus floridensis* in the stelar parenchyma of *Pinus clausa* roots (3). *S. rumicis* induced formation of syncytia, as do some species of Heteroderidae, Nacobbidae, and Rotylenchulidae (5). *S. rumicis*-induced syncytia lack cell wall ingrowths, however, as do syncytia induced by *Atalodera*, *Punctodera* spp., and *Rotylenchulus reniformis* (5,7,10).

Specimens of *S. rumicis* have been deposited in the U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland.

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