Occurrence, Parasitism, and Pathogenicity of Nematodes Associated with Sycamore (*Platanus occidentalis* L.)[1]

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Abstract: Ten species of stylet-bearing nematodes were recovered in a survey of sycamore (*Platanus occidentalis* L.) stands in Georgia. *Helicotylenchus, Xiphinema,* and *Criconemoides* were the genera found most frequently. Populations of *Hoplolaimus galeatus, Scutellonema brachyurum, Helicotylenchus dihystera* and *H. pseudorobustus* increased on greenhouse-grown sycamore, but *Trichodorus christiei, Xiphinema americanum, Meloidogyne hapla, M. arenaria* and *M. incognita* did not. *Hoplolaimus galeatus* and *S. brachyurum* are semi-endoparasites; *H. dihystera* and *H. pseudorobustus* are migratory endoparasites. *Hoplolaimus galeatus* caused extensive root necrosis and marked decrease of fresh weights of seedling roots and tops. *Helicotylenchus dihystera* and *S. brachyurum* produced only qualitatively different sparse and unhealthy root growth. *Helicotylenchus pseudorobustus* caused only a reduction in root surface area. *Key Words: Helicotylenchus, Xiphinema, Hoplolaimus, Trichodorus, Meloidogyne.*

Sycamore, *Platanus occidentalis* L., is currently being investigated as a short-rotation forest crop (6). Intensive cultivation of this species to supplement the fiber production of long-rotation timber stands may favor soil borne diseases of sycamore which is nearly disease-free in a natural woodland environment.

Information on nematode pathogens of sycamore is limited. Buhrer *et al.* (2) in 1933 reported *Meloidogyne* sp. Goeldi associated with sycamore. Springer (15) found *Hoplolaimus galeatus* (Cobb) Thorne, *Tylenchorhynchus claytoni* Steiner, and *Xiphinema americanum* Cobb associated with sycamore in New Jersey. Ruehle (13) demonstrated that *Belonolaimus longicaudatus* Rau caused damage to greenhouse-grown sycamore seedlings.

The present investigation was undertaken to identify parasitic nematodes naturally associated with sycamore, and to examine the parasitic and pathogenic capabilities of selected species on greenhouse-grown seedlings.

MATERIALS AND METHODS

SURVEY: Soil samples were taken in 1967 from ten natural or planted stands of sycamore in Georgia varying in age from 1 to 50 years. A minimum of 30 trees was selected at random within each stand. One half liter of soil was collected from the root zones of each of six-to-ten groups of three-to-five trees, by sampling to a depth of 30 cm with a 2-cm sampling tube. After samples were thoroughly mixed, a 100-cc aliquant was removed and assayed for nematodes by a centrifugal-flotation method (7). A total of 180 samples were processed, representing soil from the root zones of 730 trees.

PARASITISM: Shoot cuttings from clonal stock were rooted in a mist bed in a greenhouse and transplanted to 20-cm clay pots filled with a mixture of sandy loam and builder's sand (3:1) previously steamed 3 hr at 80 C.

Hoplolaimus galeatus, X. americanum, Helicotylenchus pseudorobustus (Steiner) Golden, *H. dihystera* (Cobb) Sher, *Tricho-*

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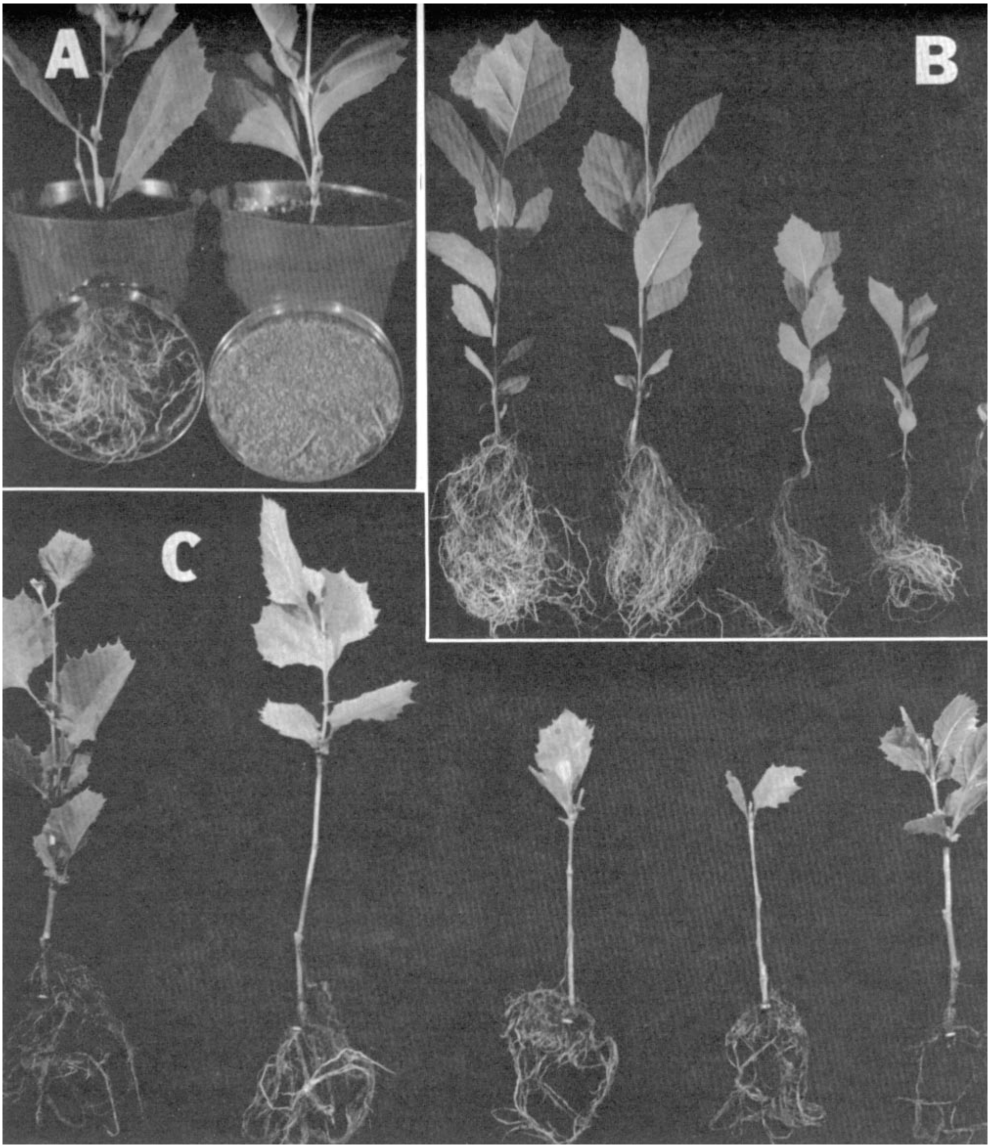

FIG. 1. Pathogenicity of *Hoplolaimus galeatus* to sycamore. **A.** Petri dish mounted over hole in side of each pot; permits roots to grow, be inoculated and examined without disturbance; **B.** Effect of *H. galeatus* on growth of sycamore seedlings after 80 days (left to right, check, supernatant, 500, 1,500, and 5,000 nematodes per pot); **C.** Effect of *H. galeatus* on growth of sycamore cuttings after 120 days (left to right, check, supernatant, 500, 2,500, and 12,500 nematodes per pot).

dorus christiei Allen, *Meloidogyne arenaria* (Neal) Chitwood, *M. hapla* Chitwood, *M. incognita* (Kofoid & White) Chitwood, and *Scutellonema brachyurum* (Steiner) Andrássy were extracted from soil using the elutriator-pan technique (11). Water suspensions (50 ml) of each species were collected with a micropipette apparatus (4) and added separately to each of nine pots containing single, rooted sycamore cuttings. Inoculum consisted of two hundred *H. pseudorobustus* or *T. christiei* per pot, whereas approximately 1,000 of each of the other species were used per pot. In addition, nine soil-filled pots with no plants were infested with nematodes in order to evaluate longevity in the absence of a host. The pots in each test were randomly arranged on greenhouse benches and watered, weeded and fertilized as needed.

Egg masses excised from tomato roots were used for inoculum in the tests of *Meloidogyne* species. Ten egg masses suspended in 50 ml of water were poured over the roots of each of nine cuttings in each test. Ten egg masses were also added to each of nine soil-filled pots containing no plants.

Three pots in each test were selected at random every three months, the seedlings removed, and the soil gently shaken from the roots. Soil adhering to the roots of each seedling was carefully rinsed into a bucket containing tap water. Four 100g aliquants were taken from the remaining soil after it had been thoroughly mixed and weighed. Both the root washings and soil aliquants were assayed separately by a centrifugal-flotation technique (7). The root and soil counts were combined to estimate the total number of nematodes in each pot. Plants inoculated with *Meloidogyne* spp. were also examined for root galls.

HISTOLOGICAL STUDY: The feeding habits of *H. pseudorobustus*, *H. dihystera*, *H. galeatus*, *S. brachyurum*, *X. americanum*, *T. christiei*, *M. hapla*, *M. incognita*, and *M. arenaria* were observed directly in the laboratory by microscopically examining excised sycamore roots. Rooted sycamore cuttings were grown in 10-cm plastic pots containing vermiculite, and a petri dish containing sand was mounted over a hole on the side of each pot to facilitate examination of roots without disturbing the root system (Fig. 1-A). Fifty nematodes suspended in tap water were poured over the roots growing in the petri dishes. At various time intervals (daily to several days) roots were excised, removed from the petri dish, washed, stained in acid fuchsin- or cotton blue-lactophenol, destained in clear lactophenol, and examined microscopically.

PATHOGENICITY: Rooted cuttings from clonal stock were prepared as in the parasitism study and planted in 20-cm clay pots. Seedlings grown from a single seed source were also used as test plants in the *H. galeatus* test. The seeds were soaked in 0.1% hydrogen peroxide for 24 hr and sown in flats containing steam-pasteurized soil. Approximately one month later, when the first true leaves had developed, the seedlings were transplanted into 10-cm clay pots containing the previously described soil mixture. *Helicotylenchus pseudorobustus*, *H. dihystera*, *H. galeatus*, and *S. brachyurum* were extracted from greenhouse pot cultures using the elutriator-pan technique. The following inoculum levels for each species were measured using a burette: *H. dihystera* 1,000, 5,000, and 25,000; *H. pseudorobustus* 500 and 12,500; *S. brachyurum* 500, 2,500, and 12,500; and *H. galeatus* on cuttings 500, 2,500, and 12,500; on seedlings 500, 1,500, and 5,000. A check treatment of 50 ml of tap water per pot, and a supernatant treatment were also included. Fifty-ml aliquants of supernatant were prepared by passing the wash water from the elutriator through a 400-mesh screen to remove all nematodes. All tests on cuttings were replicated six times and the seedling test was replicated seven times. The pots in each test were randomly arranged on greenhouse benches and watered, weeded, and fertilized as needed. After 80 days with the *H. galeatus*

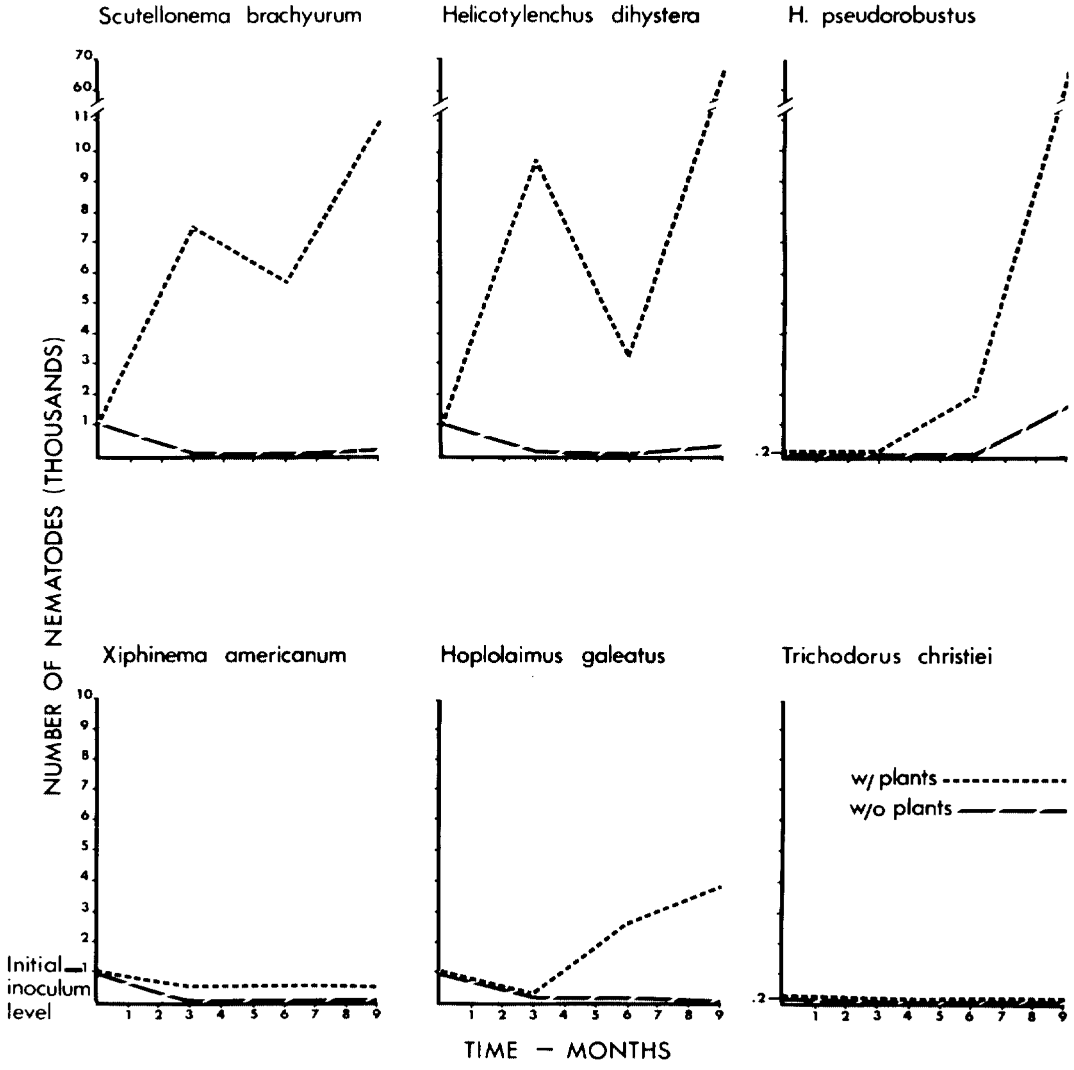

FIG. 2. Population densities of six nematode species maintained in either pots containing sycamore cuttings or fallow soil.

seedling test, 3 months with the *H. pseudorobustus* test, and 4 months with the other species, the study was terminated. Plants were removed from the pots, the roots were washed free of soil, examined, and fresh shoot and root weights recorded. An estimate of the total root surface of each cutting or seedling was obtained by determining root titration values (1). After the soil from each pot was thoroughly mixed, a 100 cc aliquant was taken and assayed for nematodes using a centrifugal-flotation method (7).

RESULTS AND DISCUSSION

SURVEY: Ten species of plant-parasitic nematodes were associated with sycamore in Georgia. At least three different genera were recovered from each stand sampled.

The soil in all stands contained one or more species of *Helicotylenchus*, with *H. pseudorobustus* occurring most often and in the highest numbers. Both *Xiphinema americanum* and *Criconemoides* sp. were found in nine of 10 stands sampled but only in low numbers (< 10 per 100 cc). *Hoplolaimus galeatus*, *H. stephanus*, *Trichodorus* sp. and *Hemicycliophora* sp. were all found in low numbers in less than half the stands sampled. *Psilenchus* sp. were recovered from the soil in four stands in moderate numbers (10–30 per 100 cc). Only *H. galeatus* and *X. americanum* have been previously reported associated with sycamore (15).

PARASITISM: Populations of *H. galeatus*, *S. brachyurum*, *H. dihystera*, and *H. pseudorobustus* increased four, 11, 70, and 350-fold, respectively, during a nine-month period, indicating that sycamore is a suitable host for each of these species (Fig. 2). Reductions in the number of *H. galeatus* after 3 months, and *S. brachyurum* and *H. dihystera* after 6 months were attributed to a drastic reduction in food availability. Accidental 2,4-D defoliation six weeks before sampling caused a reduction in the growth of these test plants. A new flush of growth and renewed vigor was evident before the next sampling and accounted for the increase in population densities.

Only *H. pseudorobustus* increased in number in the absence of a host. Between six and nine months there was an increase from an undetectable level to 2,000 nematodes per pot. Contamination from other treatments in the same test and/or the hatching of eggs of gravid females that remained inactive for six months may have caused this increase.

The population of *T. christiei* dropped to an undetectable level after three months indicating that sycamore is either an unsuitable host or the inoculum was rendered nonviable by unfavorable conditions or handling during extraction. The reduction in the population of *X. americanum* in greenhouse culture is not uncommon. The rate of increase in experimental populations is usually slow (10) and soil temperature and moisture affect reproduction (9). The test period should be extended further before valid conclusions can be drawn concerning host-parasite relationships between sycamore and *X. americanum* and *T. christiei*.

HISTOLOGICAL STUDY: Both *H. galeatus* and *S. brachyurum* penetrated the areas on fibrous roots behind the root tip extending to the region of secondary thickening. Dark lesions developed at points of entry. Both fed as semi-endoparasites with only the anterior end of the body embedded in the cortex. Frequent observations during a 2-month period failed to reveal these nematodes completely embedded in the roots. *Hoplolaimus galeatus* feeds endoparasitically on cotton (8) and pine roots (12), but it is apparently not able to enter sycamore roots completely and feeds with the body protruding. Christie (3) discussed the inability of this nematode to enter completely roots of other hosts.

Helicotylenchus dihystera and *H. pseudorobustus* penetrated fibrous roots behind the root tips and at the intersection of lateral and branch roots. Both nematodes were commonly found completely embedded in the cortical tissue. In other root sections they were found only partially embedded, a feeding habit more common for this genus (5), although *H. pseudorobustus* was reported as an endoparasite of corn (16). Females of *H. pseudorobustus* were found occasionally inside swollen root tips which were covered with root hairs, a condition unusual for this region of the root. After laying several eggs in the cortical tissue, females of this nematode were also observed with the anterior end protruding from the root surface, as if in the act of migrating back into the soil.

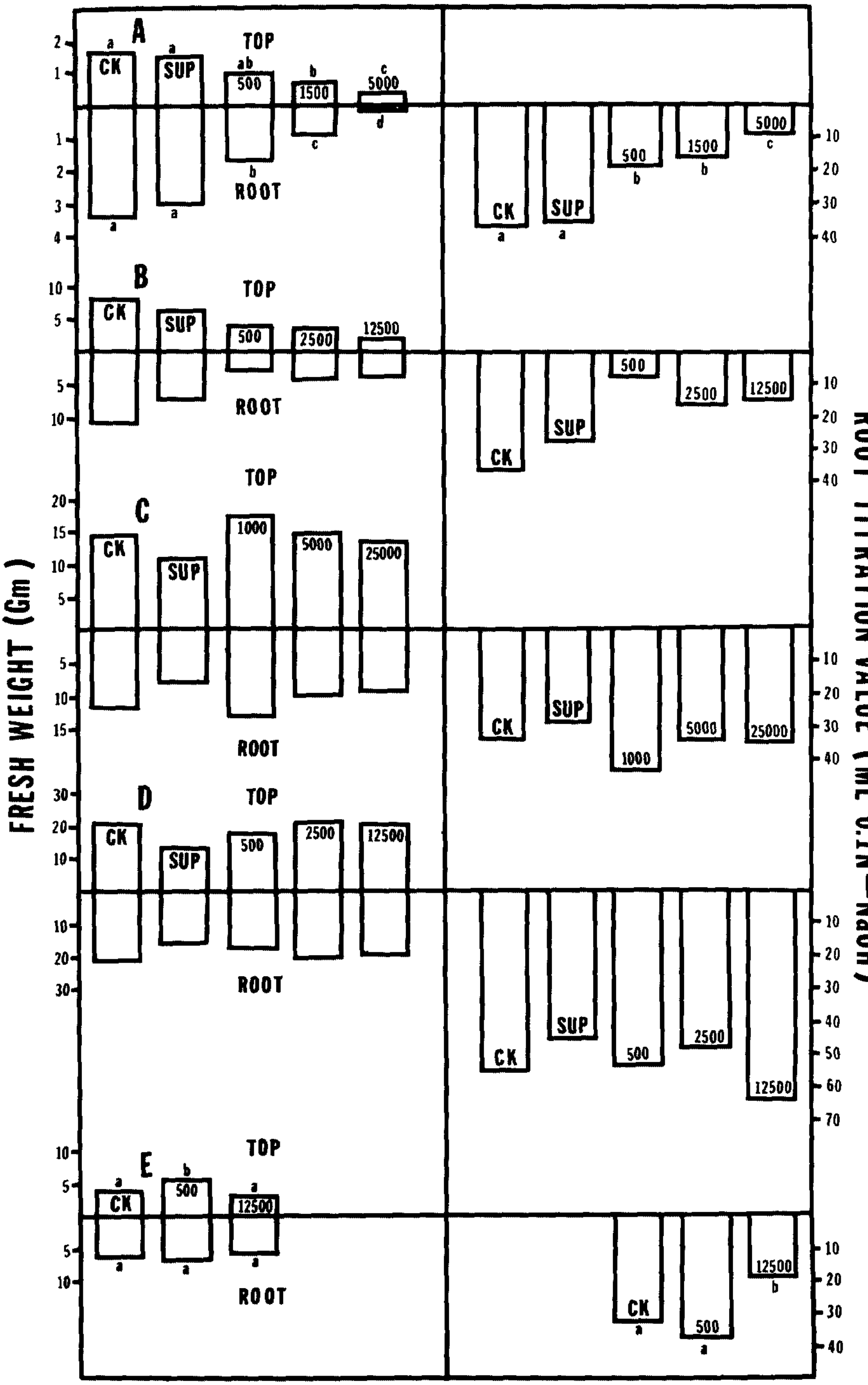

FIG. 3. Pathogenic effect of four species of plant-parasitic nematodes in terms of seedling fresh weight (tops, above line; roots, below line) and root area ($\cong$ root titration value). **A.** *Hoplolaimus*

PATHOGENICITY: *Hoplolaimus galeatus* was the most damaging of the four species tested. There was a progressive decrease in seedling growth as the inoculum level was increased (Fig. 1-B & 3-A). Seedlings receiving 2,500 and 12,500 nematodes had significantly less fresh weight and total root surface (expressed as titration values) than control seedlings receiving supernatant or tap water. Seedlings inoculated with *H. galeatus* were more severely damaged than rooted cuttings. Nematode feeding resulted in a reduction in fresh weight and total root surface of rooted cuttings which received nematodes (Fig. 1-C & 3-B), but the lack of uniformity of size in rooted cuttings within controls and treatment groups at the completion of the test accounted for the lack of statistical significance. Extensive necrosis was found on the reduced root systems of both seedlings and cuttings receiving high inoculum levels (5,000/pot with seedlings and 12,500 with cuttings).

Cuttings parasitized by *H. dihystera* (Fig. 3-C) and *S. brachyurum* (Fig. 3-D) failed to show fresh weight or total root surface differences among treatments. The roots of the control plants, both check and supernatant, were lighter in color and more fibrous than the dark, coarse root systems of the plants receiving the highest inoculum level of *H. dihystera* (25,000/pot), but a similar comparison in the *S. brachyurum* test revealed no qualitative differences.

Helicotylenchus pseudorobustus had little affect on top and root weight, and caused no root necrosis, but the higher numbers (12,500/plant) caused a significant reduction in root surface area (Fig. 3-E). Under field conditions where moisture supply, soil fertility, and temperature are often unfavorable for plant growth, this type of root injury may be sufficient to cause unthrifty plant growth.

Nematode assays of the soil and roots at the termination of these tests showed that the nematode populations had either increased or maintained the inoculum levels. No nematodes were found in the controls.

Sasser (14) reported that woody plants are preferred hosts for the four nematode species we tested on sycamore, and that they are frequently associated with declining trees and shrubs in the United States. Our results indicate that they are potential pathogens of sycamore and with intensive cultivation of this tree species chemical control may become necessary.

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galeatus on seedlings after 80 days; **B.** *H. galeatus* on rooted cuttings after 4 months; **C.** *Helicotylenchus dihystera* on cuttings after 4 months; **D.** *Scutellonema brachyurum* on cuttings after 4 months; **E.** *Helicotylenchus pseudorobustus* on cuttings after 3 months. Parameter group means with the same letter are not significantly different ($P \leqslant 0.05$).

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