Association of Nematodes and Dogwood Cankers

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Abstract: Dogwood canker is a serious production problem of unknown etiology. From May 1985 through April 1989, cankers from 290 flowering dogwood trees in 15 separate nurseries were sampled for nematodes. Seventy-three percent (213) of the cankers contained nematodes. *Panagrolaimus rigidus* (Schneider) Thorne (115/290) and *Aphelenchoides* spp. (91/290) were the most frequently collected taxa. *Panagrolaimus rigidus* was reared on 2% water agar with unidentified bacteria as the food source. *Aphelenchoides* spp. were reared in antibiotic-amended agar culture with the fungus *Glomerella cingulata* (Stoneman) Spauld. & Schrenk as a food source. Repeated attempts to culture *Aphelenchoides* spp. on dogwood callus tissue were unsuccessful. Artificially created stem wounds inoculated with combinations of *Aphelenchoides* spp. and *P. rigidus* callused completely in 60 days with no indication of canker development. Very low numbers of nematodes were recovered from inoculated trees, but *P. rigidus* and one *Aphelenchoides* sp. were efficient dispersers and occurred in treatments other than those in which they were inoculated.

Key words: aerial parasite, *Aphelenchoides* spp., *Cornus florida*, dogwood canker, flowering dogwood, hardwoods, nematode, *Panagrolaimus rigidus*, woody ornamental.

The ornamental nursery industry has become a valuable part of Tennessee's agriculture. Among row crops, only soybean, corn, and cotton have greater annual production value than nurseries. The flowering dogwood (*Cornus florida* L.) alone composes about 16% of Tennessee's annual woody ornamental production (1).

A stem canker of flowering dogwood was observed more than 25 years ago (3). It was first noticed in Virginia, but nurseries in Tennessee, North Carolina, Ohio, Maryland, and Georgia have also reported it (4–6). Incidence of cankered trees within nurseries can vary from a small percentage to 60%. The causal agent for stem canker has not been found. Lambe and Wills (5) cultured several different fungi isolated from the margin of cankers, but none produced cankers when inoculated into healthy seedlings. Treatments with several fungicides had no effect on the number or severity of the cankers.

Dogwood canker is first observed on young trees 2 to 4 years old (14). There are two different forms. One begins as a sunken area that develops on the main stem from the soil line to 1 meter up the stem, or to the first branch. At first the bark becomes roughened and cracked over a sunken area on the stem. As the canker progresses, it girdles the tree, resulting in death of branches. Often the tree is so weakened near the base that it breaks in the wind. The other form of canker is found close to the nodes and is swollen, with roughened or cracked bark. Cankered areas of bark provide oviposition sites for the dogwood borer, *Synanthedon scitula* Harris (Lepidoptera: Aegeriidae), a destructive insect pest of dogwood (9). Dogwood borer damage is often confused with dogwood canker, since it also causes cracked and roughened areas on the bark.

Santamour and McArdle (10) reported that nematodes, especially *Aphelenchoides* spp., were commonly found in dogwood cankers, and on that basis asserted that nematodes were the causal agents of dogwood canker. This assertion provided the impetus for this investigation of dogwood canker and nematodes in Tennessee nurseries. The objectives of this project were to determine if certain nematodes were consistently associated with dogwood canker and to determine whether nematodes inoculated into healthy seedlings would induce the development of dogwood cankers.

MATERIALS AND METHODS

Survey of dogwood nurseries: From May 1985 through April 1989, 290 dogwood trees with cankers from 15 nurseries in four Tennessee counties were sampled for nematodes. Trees ranged from 3 to 15 years in age, and in diameter at a 30 cm height, from 2 to 10 cm. Fifteen apparently healthy trees with similar ages and diameters were also sampled. Pieces of bark from cankers and sapwood were chipped from the trees with a hatchet and placed in jars of water for 24 hours, after which nematodes were collected on a 500-mesh (10-μm-pore) screen. Nematodes in each sample were counted. In most cases all nematodes were killed and fixed in hot 4% formalin, processed to glycerin, mounted, and labeled for subsequent identification. Nematodes from some samples were removed and cultured.

Microbivorous nematodes from several samples were reared on 2% water agar with unidentified bacteria as a food source. Cultures were transferred approximately every 3 weeks.

Stylet-bearing nematodes from 14 samples were transferred to potato dextrose agar (PDA) amended with 10 ml/liter of a 0.9% NaCl solution containing penicillin (5,000 U/ml), streptomycin (5 mg/ml), and neomycin (10 mg/ml), with the fungus *Glomerella cingulata* (Stoneman) Spauld. and Schrenk as a food source. To maintain bacteria-free cultures of stylet-bearing nematodes, 5–10 nematodes were hand-picked from each culture and placed in a BPI watch glass containing a 1.0% Thimerosal (sodium ethylmercurithiosalicylate) solution. After incubation for 15 minutes, the nematodes were transferred to a 5-ml beaker of streptomycin-penicillin-neomycin-amended, sterile distilled water for 5 minutes, then to a 5-ml beaker of sterile distilled water for 5 to 15 minutes. Nematodes were transferred to petri dishes containing PDA, which was observed for bacterial colony development. If no bacteria appeared after 7 days, dishes were seeded with *G. cingulata* for nematode feeding and reproduction.

Inoculation of dogwood trees: Two-year-old, bare-root, budded pink dogwood trees were potted in 4-liter containers in a sandy loam (artificial soil mix) and grown in the greenhouse for 2 years. During the fall of the 3rd year, 50 trees were moved outside under 60% shade and transplanted to 11-liter containers filled with a noncomposted pine bark mix.

The following spring, the two most commonly collected species of *Aphelenchoides* (here designated A1 and A2), *Panagrolaimus rigidus* (Schneider) Thorne (Pr), and *Eucephalobus* sp. (E) were collected from stock cultures for tree inoculations. The *Eucephalobus* sp. originally had been undetected in collections from cankers on nursery trees but was found in *P. rigidus* cultures as a minor constituent. The two *Aphelenchoides* spp. differed in lip region profile and tail terminus shape (Fig. 1). The *Aphelenchoides* spp. were collected on a 500-mesh (10-μm-pore) screen and concentrated with centrifugation for 1 minute in 15-ml centrifuge tubes. They were counted, then rinsed twice in streptomycin-penicillin-neomycin-amended sterile distilled water and once in sterile, distilled water. *Panagrolaimus rigidus* + *Eucephalobus* sp. were concentrated with centrifugation, counted, and rinsed in sterile distilled water. A1 and A2 were resuspended in sterile distilled water to produce an inoculum level of 4,000 nematodes/ml. The microbivores were resuspended in sterile distilled water to produce an inoculum level of 2,000 nematodes/ml. For the two combined nematode treatments (A1 + Pr + E and A2 + Pr + E), a total inoculum level of 6,000 nematodes per ml was used. Forty-nine trees were randomly arranged in a 7 × 7 Latin Square design. The seven treatments were as follows: 1) A1 only, 2) A2 only, 3) Pr + E, 4) A1 + Pr + E, 5) A2 + Pr + E, 6) supernatant control, and 7) distilled water control. The supernatant control consisted of the combined nematode-free supernatant from A1, A2, and microbivore samples.

Inoculations were performed by cutting a small notch into the cambium of the tree between 8 and 14 cm from the base of the
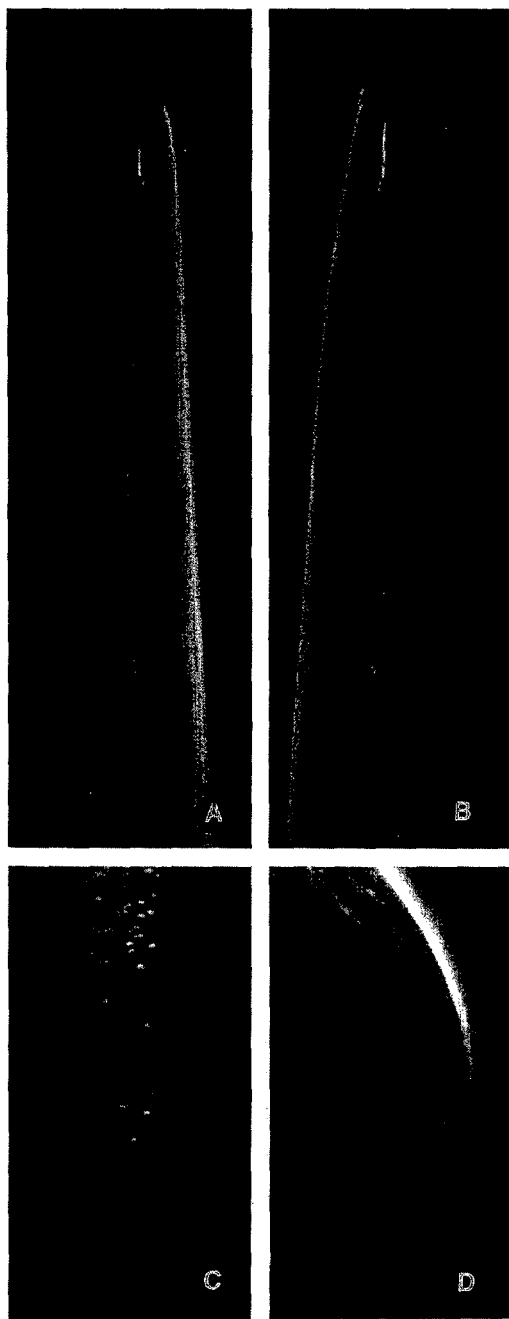
FIG. 1. Anterior and posterior regions of *Aphelenchoides* taxa used in inoculation studies. A,C) *Aphelenchoides* sp. no. 1 (A1). B,D) *Aphelenchoides* sp. no. 2 (A2). Scale = 10 μm.

stem and inserting a sterile 1-cm² piece of cotton gauze into the wound. A 0.25-ml aliquot of each suspension was pipetted onto the gauze. The wound was closed over the gauze, wrapped with Parafilm® (American National Can, Greenwich, CT),

and sealed with tape to prevent evaporation. The inoculated trees were kept outside under 60% shade.

After 60 days the gauze and parafilm were removed, and the trees were felled. A 5–7 cm long section of stem including the wounded area was placed in a jar of water for 24 hours. Nematodes were collected on a 500-mesh (10-μm-pore) screen and identified to genus. In the case of *Aphelenchoides* spp., nematodes were separated into taxa on the basis of morphological characters.

Development of Aphelenchoides *sp. on dogwood callus:* One hundred dogwood seeds were cold-stratified for 4 months, surface-sterilized with 1.05% NaOCl, and placed on 2% water agar to germinate. Upon germination, the epicotyl was excised and placed on Schenck and Hildebrandt (11) medium amended with 2 μM N-(phenylmethyl)-1H-purine-amine (6-benzylaminopurine; BA) and 1 μM 2,4-dichlorophenoxyacetic acid (2,4-D). Subsequent calli were cultured through two transfers and grown to 2 cm in diameter.

Aphelenchoides sp. females were surface-sterilized with a 1.0% Thimerosal solution as described above. Five nematodes were pipetted into each of two cultures of callus tissue. The cultures were incubated at 26 C for 30 days, then examined for nematode reproduction and development.

RESULTS

Survey of dogwood nurseries: Two hundred thirteen of the 290 canker samples (73%) contained nematodes (Table 1); the 15 samples from healthy trees contained no nematodes. *Aphelenchoides* spp. and *P. rigidus* occurred most often, and they frequently occurred together in the same canker.

Several species of *Aphelenchoides* were found, but most could not be reliably identified because of the large number of incomplete and overlapping species descriptions of known species. Nematodes from one sample were identified as *A. bicaudatus* (Imamura) Filipjev and Schuurmans Stekhoven (12) by virtue of the distinctive bifurcate tail terminus, stylet length (9.5

TABLE 1. Nematode taxa extracted from dogwood cankers.

Taxon	Number of infested cankers (N = 290)
Aphelenchoides bicaudatus (Imamura) Filipjev & Schuurmans-Stekhoven	1
Aphelenchoides spp.	90
Chambersiella rodens Cobb	1
Deladenus sp.	1
Eucephalobus sp.	†
Eumonhystera sp.	2
Macrolaimus sp.	1
Panagrolaimus rigidus (Schneider) Thorne	115
Paraphelenchus sp.	2

† Small numbers of *Eucephalobus* sp. were found in *P. rigidus* cultures.

µm), vulva position (68%), and two lateral incisures. The other *Aphelenchoides* specimens were divided into five morphotaxa on the basis of lateral incisure number, lip region profile, and tail terminus shape.

Inoculation of dogwood trees: Artificial inoculation wounds on all of the trees were completely closed by plant callus (Fig. 2B,C). Some of the trees even callused over the gauze to the point that it could not easily be removed (Fig. 2C). There was lit-

tle variation among treatments, and none of the trees exhibited any signs of canker formation.

The overall numbers of nematodes recovered per treatment were very low compared to the initial inoculum levels (Fig. 3). A1 was recovered at much lower levels than A2 and was found only in the two treatments in which it was included initially. A2 was recovered from every treatment, including the controls, except the Pr + E treatment. *Panagrolaimus rigidus* was recovered from all treatments, but no *Eucephalobus* sp. were recovered from any treatment.

Development of Aphelenchoides sp. on dogwood callus: Of the 10 *Aphelenchoides* sp. females placed on dogwood callus tissue, 4 produced eggs that later hatched. After 30 days, all of the females and juveniles were dead, and no more eggs were observed.

DISCUSSION

Our knowledge of nematodes found in the above-ground portions of hardwood trees is scant. Lehmann (7) reported isolations of *Aphelenchoides ritzemabosi* (Schwartz) Steiner and Buhrer and *Pana-*
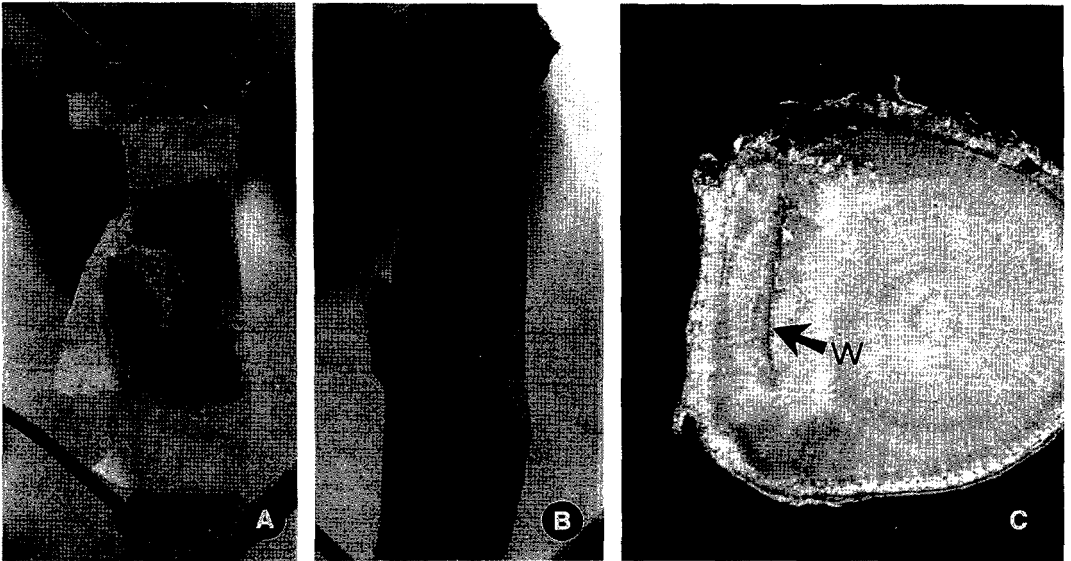

FIG. 2. Response of dogwood to wound inoculation with nematodes. A) Inoculation method—parafilm partially removed to show position of gauze. B) Completely callused wound 60 days after inoculation. C) Cross-section of completely callused stem 60 days after inoculation (W = wound area).
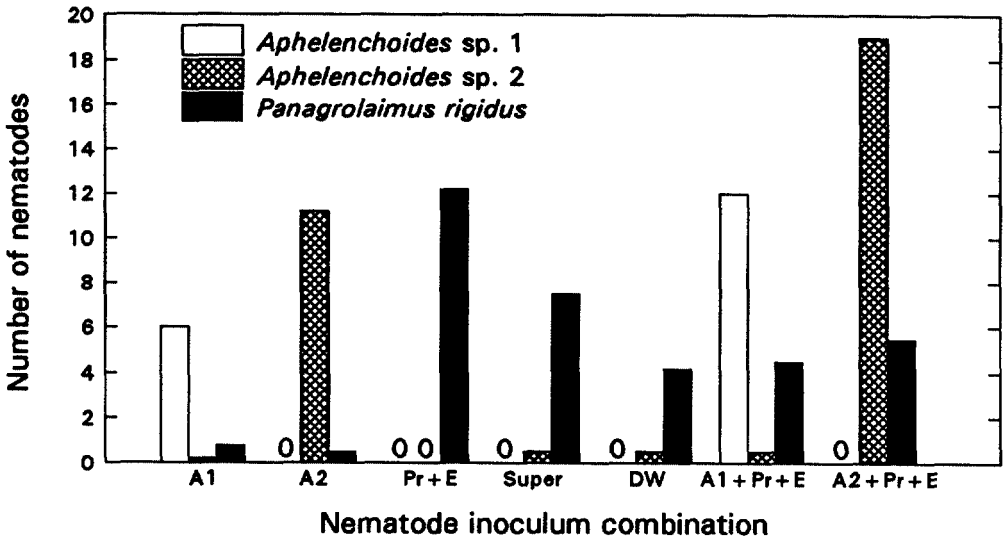
FIG. 3. Nematodes in experimentally wounded dogwood tissue and their dispersal to dogwood wounds 60 days after inoculation. A1 = *Aphelenchoides* sp. no. 1; A2 = *Aphelenchoides* sp. no. 2; Pr + E = *Panagrolaimus rigidus* + *Eucephalobus* sp.; Super = nematode-free supernatant from nematode culture extractions; DW = distilled water. Each bar is the mean of seven replicates.

grolaimus rigidus (Schneider) Thorne from branch portions of an elderberry that exhibited symptoms of witches' broom. In eastern Austria, Tomiczek (13) isolated a species of *Bursaphelenchus* resembling *B. mucronatus* from the twigs and trunks of white oak with symptoms of oak decline. The greatest numbers of nematodes were found in association with the fungus *Colpoma quercinum* (Fr.) Wallr. Massey (8) found *A. rhytium* Massey on pignut hickory in association with the bark beetle *Chramesus hicoriae* Leconte.

To date the most extensive survey of nematodes associated with the stem malformations of hardwood trees was conducted by Santamour and McArdle (10). They isolated a species of *Aphelenchoides* resembling *A. fragariae* (Ritzema Bos) Christie and *Panagrolaimus subelongatus* (Cobb) Thorne from cankered dogwood trees and postulated that they were the causal agents of the malformations. *Panagrolaimus subelongatus* was isolated from burls of sugar maple (*Acer saccharum* March.), and both nematode species were isolated from burls of red maple (*Acer rubrum* L.), Higan cherry (*Prunus subhirtella* Mig.), red oak (*Quercus rubra* L.), black lo-

cust (*Robinia pseudoacacia* L.), and Siberian elm (*Ulmus pumila* L.).

In our study, nematodes were found in cankers on most, but not all, trees. If nematodes indeed were the sole cause of dogwood canker, then the first of Koch's postulates would require that they be consistently present. However, a substantial minority of cankers (27%) was found to be devoid of nematodes, thereby not fulfilling this first postulate. It is still possible that nematodes incited cankers, which then became separately infected with an unknown necrotrophic organism. Repeated efforts by a number of workers to isolate causal agents (3,4) and then reinfect trees have consistently failed, calling into doubt any role for nematodes as wounding agents.

When dogwood trees were inoculated with one or more mixtures of these species of nematodes, inoculation wounds were completely callused after 60 days. The development of healthy callus tissue at the wound site of all inoculated trees is another indication that nematodes are not the causal agent of dogwood canker. Environmental conditions and tree vigor may have affected the rate of callus formation. Trees were inoculated in May, when

growth is rapid and wound repair is likely to be faster. If trees had been inoculated in late summer, autumn, or winter, wound closure might have been delayed. The effect of such delays on nematode population dynamics was not studied in this experiment. The rapid decline of nematode population densities in fresh wounds, however, provides substantial evidence that nematodes are not the major incitants of dogwood canker.

Another indication that *Aphelenchoides* spp., in particular, are not the causal agents of dogwood canker was the failure to successfully establish them on dogwood callus in the laboratory. Although certain aphelenchoid plant parasites can be reared on plant callus (2), none of the *Aphelenchoides* spp. in this study survived on dogwood callus, further evidence that these species are unlikely etiological candidates. The Aphelenchida are characteristic inhabitants of rotting wood, insect galleries, and frass (8). The common presence of nematodes in cankers probably is due to the availability of fungal hyphae for nutrition.

The tree inoculation study demonstrated some rather distinct differences in the dispersal abilities of the nematode species used. A2 was able to spread to trees in most of the other treatments, whereas A1 was found only on trees within its own treatments. *Panagrolaimus rigidus* also dispersed readily among the trees. The mechanisms by which these nematodes moved to other trees was not readily apparent. Since the period during the tree inoculation study was usually rainy, rainfall, splashing, and wind may have served as dispersing mechanisms. Because of space limitations, the inoculated trees were arranged in such a manner that overlapping foliage did occur, so it was possible for nematodes to swim from tree to tree when the leaves were wet. Nematodes could also have spread from pot to pot by splashing rain, followed by migration up the stem to a favorable feeding site such as the inoculation wound.

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