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Association of nematodes and dogwood cankers

Louann H. Self

To the Graduate Council:

I am submitting herewith a thesis written by Louann H. Self entitled "Association of nematodes and dogwood cankers." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Ernest C. Bernard, Major Professor

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Date 30 November 1989

ASSOCIATION OF NEMATODES AND DOGWOOD CANKERS

Presented for the

Master of Science

Degree

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Louann H. Self

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ABSTRACT

Dogwood canker is a serious production problem of unknown cause. From May 1985 through April 1989, cankers from 290 flowering dogwood trees in fifteen separate nurseries were sampled for nematodes. Seventy-three percent (213) of the cankers were found to contain nematodes. Panagrolaimus rigidus and Aphelenchoides spp. were reared in the laboratory on antibiotic media with Glomerella cingulata as a food source, and Panagrolaimus rigidus was reared on water agar with bacteria as a food source. Repeated attempts to culture Aphelenchoides sp. on dogwood callus tissue were unsuccessful. When dogwood trees were inoculated with one or a mixture of Aphelenchoides spp. or P. rigidus, inoculation in wounds was completely callused after 60 days with no indication of canker development.

Very low levels of nematodes were recovered from the inoculated trees, but P. rigidus and one Aphelenchoides sp. showed a high degree of dispersability by occurring in treatments other than those in which they were inoculated.

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CHAPTER I

INTRODUCTION

The ornamentals nursery industry has become a valuable part of Tennessee's agriculture. Among row crops only soybeans, corn, and cotton have greater annual production value than nurseries. The flowering dogwood (Cornus florida L.) alone comprises about 16% of Tennessee's annual woody ornamental production (Badenhop, et al. 1985).

A stem canker of flowering dogwood was observed about twenty years ago (Lambe 1977). It was first noticed in Virginia (Lambe and Wills 1980), but nurseries in Tennessee, North Carolina, Ohio, Maryland, and Georgia have also reported it (Gouin 1976, Lambe and Wills 1980a, Lambe and Wills 1980b, Lambe and Wills 1981). Incidence of cankered trees within nurseries has varied from a small percentage to 60%. A number of different cankers occur on flowering dogwood, but the causal agent for stem canker has not been found. Botryosphaeria dothidea (ribis) has been reported as a canker fungus, occasionally causing dieback, and killing branches and whole trees in the northeastern United States. Nectria galligena causes a zonate canker in which tiny red, balloon-shaped perithecia develop during wet weather (Lambe and Jones 1982). Lambe and Wills (1980) cultured several different fungi isolated from the margin

of cankers, but none were observed to produce 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 two to four years old (Windham and Montgomery 1988). 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 will break easily 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), the most destructive insect pest of dogwood (Santamour and McArdle 1987). Larvae of the borer can girdle the tree as they feed. Dogwood borer damage is often confused with the dogwood canker since it also causes cracked and roughened areas of the bark.

Santamour and McArdle (1987) hypothesized that nematodes were the causal agents of dogwood canker, based on field studies at the U.S. National Arboretum. Their inconclusive results provided the basis for this

investigation of dogwood canker in Tennessee nurseries which consisted of four objectives: 1) to survey dogwood cankers for nematodes; 2) to determine identities of nematodes found in cankered tissue; 3) to rear stylet-bearing species, especially aphelenchoid species, associated with cankers; 4) to inoculate healthy seedlings with laboratory-reared nematodes and determine the extent of canker development.

CHAPTER II

LITERATURE REVIEW

Our knowledge of nematodes found in the above ground portion of hardwood trees is scant. Lehmann (1963) reported isolations of Aphelenchoides ritzemabosi (Schwartz) Steiner and Buhrer and Panagrolaimus rigidus (Schneider) Thorne from branch portions of an elderberry that exhibited symptoms of witches broom. In eastern Austria Tomiczek (1988) isolated a species of Bursaphelenchus resembling B. mucronatus from the twigs and trunks of white oak with symptoms of oak decline. The greatest number of nematodes were found in association with the fungus Colpoma quercinum (Fr.) Wallr. Massey (1974) 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 (1987). 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 agent. P. subelongatus was isolated only from burls of sugar maple (Acer saccharum Marsh.) 'Green Mountain' 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 locust (Robinia pseudoacacia L.), and Siberian elm (Ulmus pumila L.).

CHAPTER III

MATERIALS AND METHODS

Survey of Dogwood Nurseries

From May 1985 through April 1989, 290 cankered dogwood trees from fifteen nurseries in four counties were sampled for nematodes. Trees ranged from three to fifteen years in age, and in diameter from 2 cm-10 cm. Fifteen apparently healthy trees were also sampled.

Pieces of cankered bark and sapwood were chipped from the trees with a hatchet and placed in jars of water for 24 hours. Nematodes were collected on a 500 mesh (10 µm pore) screen. Nematodes in each sample were counted, killed and fixed in hot 4% formalin, processed to glycerin (Seishorst 1959), mounted, and labeled for subsequent identification. Stylet-bearing nematodes from fourteen samples were transferred to potato dextrose agar amended with 10 ml/L of a 0.9% NaCl solution containing penicillin (5000 U/ml) streptomycin (5 mg/ml), and neomycin 10 mg/ml with Glomerella cingulata (Stoneman) Spauld and H. Schenk as a food source. Microbivorous nematodes were reared on 2% water agar with bacteria as a food source.

In order to maintain bacteria-free cultures of stylet-bearing nematodes, five to ten 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 potato dextrose agar and observed for bacterial development. If no bacteria appeared after 7 days, plates were seeded with G. cingulata.

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. The third fall 50 trees were moved outside under 60% shade and transplanted to 11 liter containers in a noncomposted pine bark mix.

The following spring two morphologically differing species of Aphelenchoides (here designated AI and AII), Panagrolaimus rigidus (Schneider, 1886) Thorne, 1937, and an unidentified species of Cephalobidae were prepared for tree inoculations. 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 and the cephalobids were concentrated with centrifugation, counted, and rinsed in sterile distilled water. AI and AII were resuspended in sterile distilled water to produce an inoculum level of 4,000 nematodes per ml. The microbivores were resuspended in sterile distilled water to produce an inoculum level of 2,000 nematodes per ml. For the two combined nematode treatments (AI + P. rigidus + Cephalobidae and AII + P. rigidus + Cephalobidae) an inoculum level of 6,000 nematodes per ml was used. The supernatant from AI, AII, and the microbivores was reserved for use as a control inoculation.

Forty-nine trees were inoculated and randomly arranged in a 7x7 Latin Square design. The seven treatments were as follows: 1) Aphelenchoides I only; 2) Aphelenchoides II only; 3) Panagrolaimus rigidus + Cephalobidae; 4) Aphelenchoides I + Panagrolaimus rigidus + Cephalobidae; 5) Aphelenchoides II + Panagrolaimus rigidus + Cephalobidae; 6) Supernatant Control; 7) Distilled Water Control.

Inoculations were performed by cutting a small notch into the cambium of the tree between 8 and 14 cm from the base of the stem, and inserting a sterile piece (1 cm^2) 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, and sealed

with tape to prevent evaporation (page 14). The inoculated trees were kept outside under 60% shade.

After 60 days the trees were cut, and a 5-7 cm section 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 identified to species-level taxa.

Development of Aphelenchoides sp. on Dogwood Callus

One hundred Cornus florida L. seeds were cold-stratified for 3 months, surface-sterilized with 1.05% NaOCl, and placed on 2% water agar to germinate. Upon germination, the epicotyl was excised and placed on Schenk and Hildebrandt (1972) medium amended with 2 µm BAP and 1 µM 2,4-D. After 4 days undifferentiated callus cells were observed. The calli were cultured through two transfers and grown to 2 cm in diameter.

Ten Aphelenchoides sp. females were surface-sterilized with a 1.0% Thimerosal solution, as described earlier, and placed (5 each) on two cultures of callus tissue with a sterile glass pipet. The cultures were incubated at 26° C for 30 days, then examined for nematode reproduction and development.

CHAPTER IV

RESULTS

Survey of Dogwood Nurseries

Two hundred thirteen of the 290 canker samples (73%) were found to contain nematodes (Table 1), and the 15 samples from healthy trees contained no nematodes. The genera Eumonhystera and Paraphelenchus were each found in two samples, and Macrolaimus, Chambersiella, and Psilenchus were each found in one sample. Aphelenchoides and Panagrolaimus occurred most often, and frequently occurred together.

Several species of Aphelenchoides were found, but most could not be reliably identified with known species due to the large number of incomplete and overlapping species descriptions (W. R. Nickle, personal communication). Nematodes from one sample were determined to be A. bicaudatus (Imamura, 1931) Filipjev and Shuurmans Stekhoven, 1941 (Siddiqui and Taylor 1967) by virtue of the very distinctive bifurcate tail termini, stylet length (9.5μ), vulva position (68%), and number of lateral incisures (2). The remaining Aphelenchoides species found could be divided into two groups: those with three lateral incisures, and those with four. Within those two groups the most obvious morphological difference was the tail

Table 1. Nematodes Extracted from Dogwood Cankers.

Genus	Number of Trees (of 290)
Panagrolaimus	115
Aphelenchoides	91
Eumonhystera	2
Paraphelenchus	2
Deladenus	1
Macrolaimus	1
Chambersiella	1

terminus (Figure 1F-J). Three distinct types were observed; a simple mucro (Figure 1F), a star-shaped mucro (Figures 1G and 1H), and a terminal peg with minute brush-like processes (Figures 1I and 1J). One species has a wider tail, narrower stylet, and a lip region less offset than the others. The two taxa illustrated with star-shaped tail termini have different tail widths and the head offset to varying degrees (Figures 1G and 1H).

Inoculation of Dogwood Trees

All of the trees that were inoculated, including the controls, formed callus tissue at the wound site (Figure 2C). Some of the trees even callused over the gauze to the point that it could not easily be removed (Figure 2B). Wounds on all trees were completely callused regardless of treatment. There was little 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 (Figure 3). AI was recovered at much lower levels than AII and was found only in the two treatments in which it was included initially. AII was recovered from every treatment, including the controls, except the P. rigidus and Cephalobidae treatment. P. rigidus was recovered from all treatments, but no Cephalobidae were recovered from any

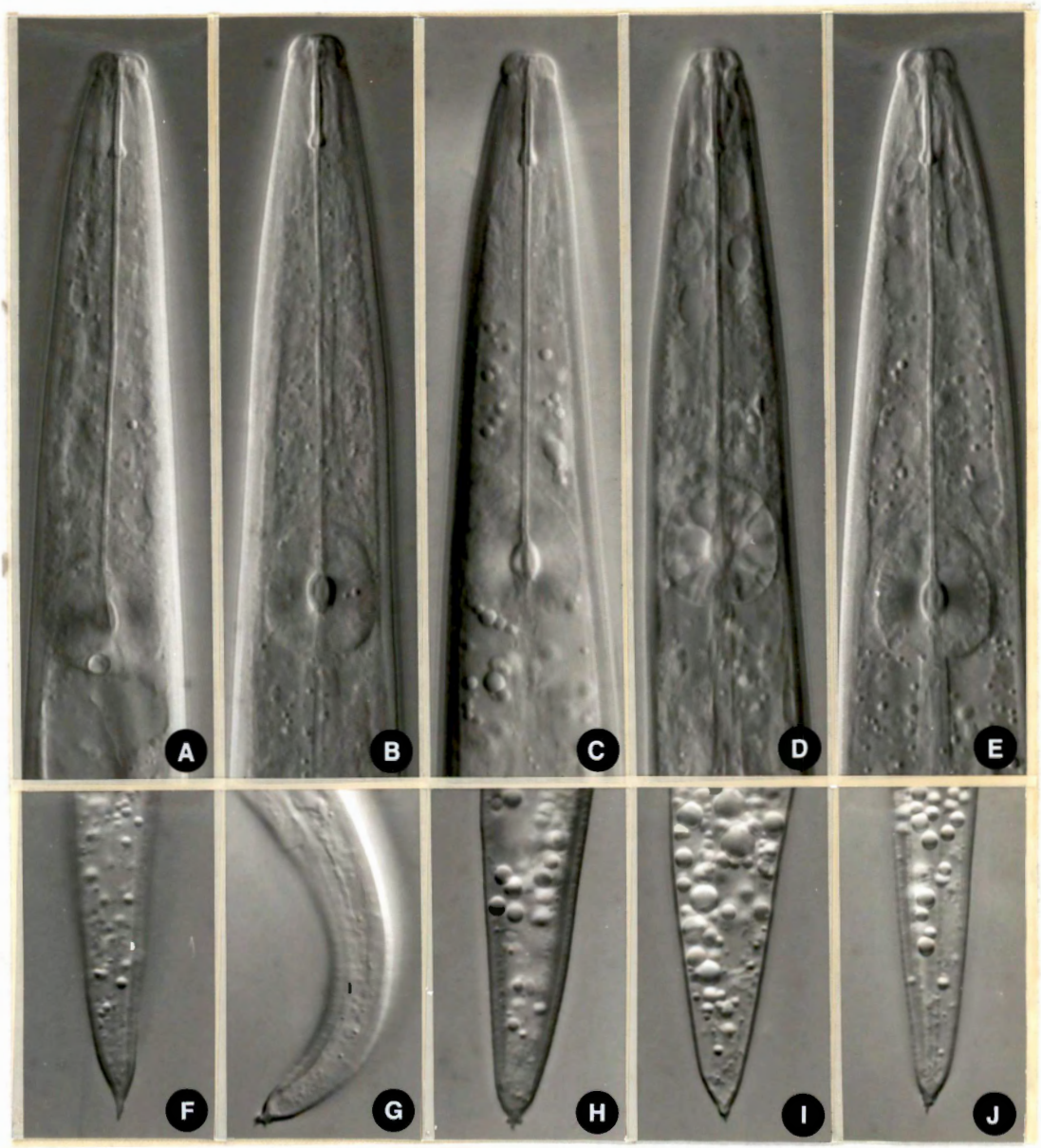

Figure 1. Representative examples of Aphelenchoides species cultured in this study: A-E, Anterior ends; F-J, Tail tips. A,F) Aphelenchoides sp. I (AI); B,G) Aphelenchoides sp. II (A II); C,H) Aphelenchoides sp. III; D,I) Aphelenchoides sp. IV; E,J) Aphelenchoides sp. V. Scale = 20 µm.

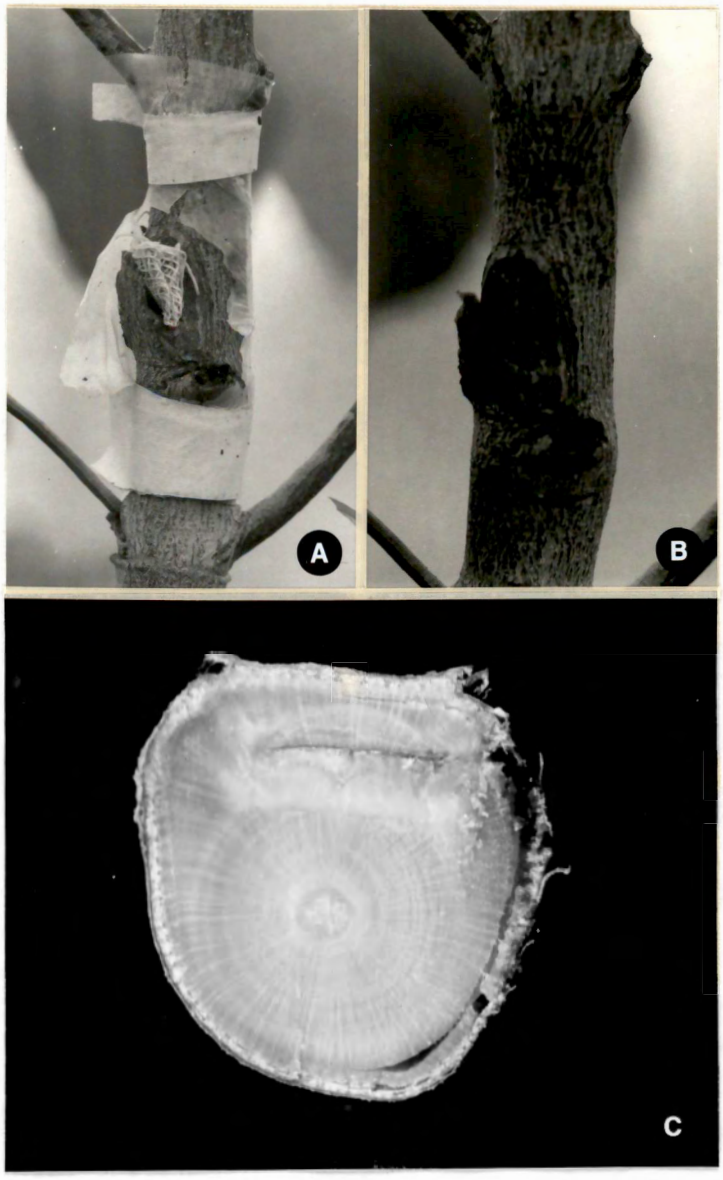

Figure 2. Response of dogwoods to wound inoculation with nematodes. A. Inoculation method: Parafilm partially removed to show position of gauze. B. Completely callused wound 60 days after inoculation. C. Cross-section of completely callused stem 60 days after inoculation.

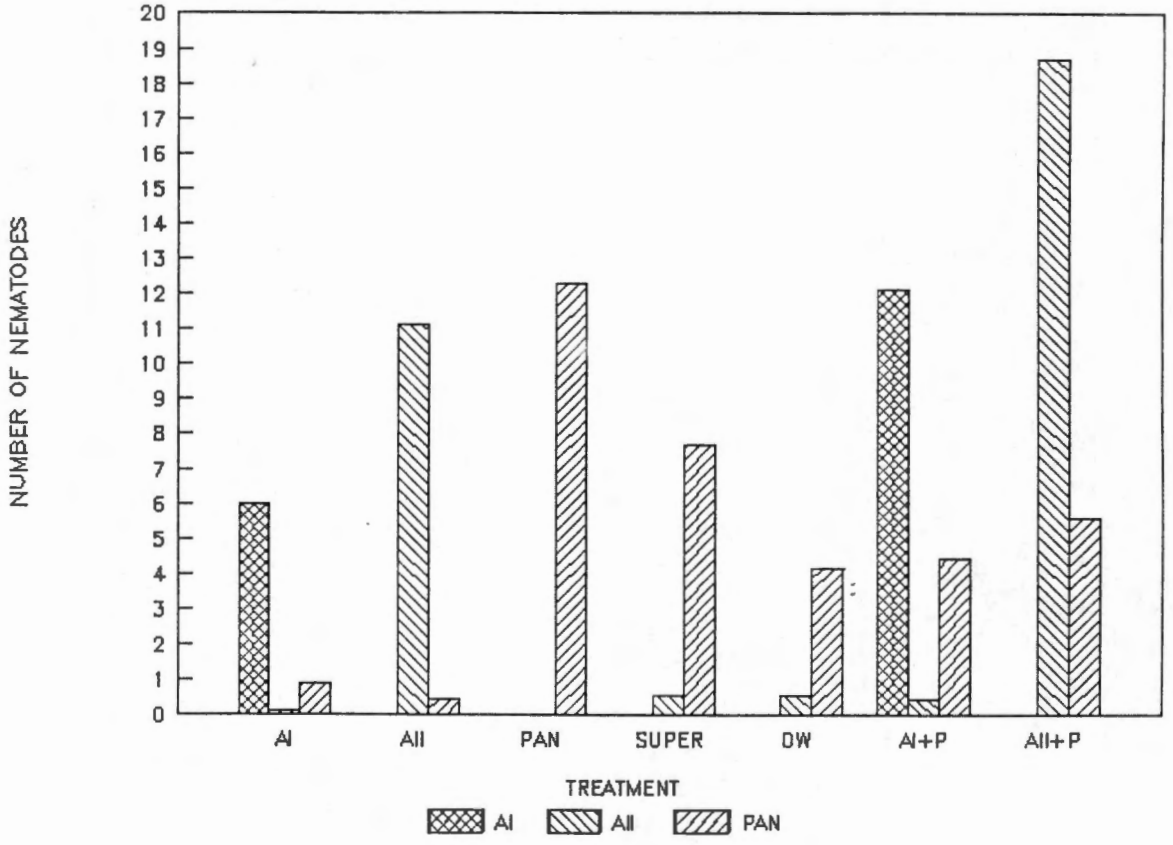

Figure 3. Nematodes extracted from artificial wounds on dogwood 60 days after nematode inoculation. Each bar represents the mean of seven trees. Treatments: AI = Aphelenchoides sp. I, initial inoculum (P_i) = 1,000 nematodes/wound; AII = Aphelenchoides sp. II, P_i = 1,000 nematodes/wound; AI+P = Aphelenchoides sp. I+ P. rigidus + Cephalobidae, P_i = 1,500 nematodes/wound; Pan. = P. rigidus + Cephalobidae, P_i = 500 nematodes/wound; Supernatant = Supernatant control from nematode cultures; DW = Distilled water control.

treatment. Both Aphelenchoides species were recovered at higher levels when inoculated in combination with P. rigidus.

Development of Aphelenchoides sp. on Dogwood Callus

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

CHAPTER V

DISCUSSION

Nematodes were found in cankers on most, but not all, trees. Santamour and McArdle (1987) suggested that Aphelenchoides spp. caused dogwood cankers. 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 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 (Lambe 1977, Lambe and Wills 1980a) and then reinfect trees have failed uniformly, calling into doubt any role for nematodes as wounding agents.

When trees were inoculated with one or a mixture 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 had an effect on 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 these experiments. The rapid decline of nematode populations in fresh wounds 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 (Dolliver, Hildebrandt, and Riker 1962), none of the Aphelenchoides spp. cultured on fungi in this study survived on dogwood callus, further evidence that species used in this study are unlikely etiological candidates. The Aphelenchida are characteristic inhabitants of rotting wood, insect galleries, and frass (Massey 1974). The common presence of nematodes in cankers is almost certainly due to the availability of fungal hyphae for nutrition.

The tree inoculation study demonstrated some rather distinct differences in the dispersability of the nematode species used. Aphelenchoides sp. II was able to spread to most of the other treatments, whereas Aphelenchoides sp. I was found only within its own treatments. P. rigidus also

showed a high degree of dispersability. The mechanisms by which these nematodes moved to other trees is not readily apparent.

A. ritzemabosi, a leaf parasite of chrysanthemum, migrates up stems when the plant is wet with rain or dew and infects the lower leaves by entering the stomata. A. besseyi, a strawberry parasite, similarly moves over surface of plants when they are wet with rain or dew, and may spread from plant to plant by means of touching foliage. Christie (1959) also states that nematodes may be washed off the plants and over the surface of the soil during heavy rains. Since the period during the tree inoculation study was unusually rainy, rainfall, and even splashing, 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.

Another possible means of dispersal is insect vectoring. Massey (1974) reported at least five species of Aphelenchoides associated with bark beetles in the United States. If an insect vector were involved in nematode dispersal, it would help explain the relatively high number

of cankers from which nematodes were isolated. The dogwood borer is often found in association with dogwood cankers and would be a likely candidate for a nematode vector but has not been studied as such to this point.

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VITA

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Ms. Self is a member of the Society of Nematologists, the American Phytopathological Society, and the American Horticultural Society. She is employed by the Tennessee Department of Agriculture as a nematologist.