Association of Nematodes with Dogwood Canker and Stem Malformations on Other Trees[1]

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Abstract

Two nematodes, one stylet-bearing and allied to the *Aphelenchoides fragariae* complex and the non-stylet species *Panagrolaimus subelongatus*, were isolated from the disrupted bark on main trunks and branches of young flowering dogwood (*Cornus florida* L.) trees exhibiting symptoms of "dogwood canker" disease. Similar nematodes were isolated from burls on the trunks of Siberian elm (*Ulmus pumila* L.), Higan cherry (*Prunus subhirtella* Miq.), red oak (*Quercus rubra* L.) and black locust (*Robinia pseudoacacia* L.), whereas only the *Panagrolaimus* species was found in abnormal tumorous growths on the trunks of the Green Mountain cultivar of sugar maple (*Acer saccharum* Marsh. 'Green Mountain'). Nematodes were recovered 2 months after inoculation into young dogwood stems in June, 1983 but none could be detected after the inoculated trees had been subjected to winter temperatures as low as −22°C (−4°F) even though nematodes in established cankers survived under the same conditions. Inoculations made in July, 1984 into callus tissue developed after wounding in 1983 or 1984 produced canker-like symptoms but the nematodes were not reisolated in 1985. The total number of nematodes per canker was low and older cankers often yielded no nematodes, even though the canker persisted.

Index words: *Cornus, Aphelenchoides, Panagrolaimus*, tumor, burl, *Acer, Ulmus, Robinia, Prunus*, maple, elm, black locust, cherry

Introduction

Dogwood canker is an important problem in the production and landscape use of our native flowering dogwood (*Cornus florida* L.). Abnormal localized trunk swelling and bark disruption render affected plants unsalable and unsuitable for planting. Stems frequently break off at the cankered area (Fig. 1) and the canker is a favorable site for attack by the dogwood borer (*Synanthedon scitula* (Harris).

Often, a high percentage of young, visually symptomless dogwoods purchased by northern growers from southern nurseries developed cankers after 1 or 2 years in the field. The cause of dogwood canker is unknown.

We first became concerned with dogwood canker in November, 1982, during the time that the U.S. National Arboretum was engaged in collecting millions of flowering dogwood seed from throughout the species range for shipment to Japan as part of a dogwood-cherry seed exchange program. We received a letter from a horticulturist in Ohio warning us that this program could be responsible for in-

Fig. 1. **Cankers on stems of flowering dogwood.**

[1]Received for publication November 2, 1986; in revised form May 12, 1987.

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[3]We would like to thank Dr. E.C. Bernard (Univ. Tenn., Knoxville) for his thoughtful review of an earlier version of this manuscript in May, 1985 and for his already published (6) confirmation of the dogwood canker-nematode association. The authors are indebted to Dr. R.G. Lambe (VPI & SU, Blacksburg, VA) for samples of cankered stems and for living trees with and without cankers. Drs. A.M. Golden and W.R. Nickle (USDA, ARS, Beltsville, MD) were most helpful in providing advice on nematode idntification and culture and in preparing specimens of *Panagrolaimus* for identification by Dr. R.V. Anderson (Biosystematics Research Institute, Agriculture Canada, Ottawa). Seedlings and cultivars of dogwood were contributed by Ingleside Plantation Nurs., Oak Grove, VA; Boyd Nurs. Co., McMinnville, TN; and Commercial Nurs. Co., Decherd, TN. W.N. Wandell (IL) and Princeton Nurs. (NJ) cooperated in supplying samples of sugar maple "tumors."

troducing a new disease, dogwood canker, into Japan, because that malady was seed borne. This contention was based on his observations that the proportion of cankered dogwoods varied greatly among nursery sources, and was probably related to seed source.

However, there was no literature pertinent to this subject and conversations with researchers familiar with dogwood canker convinced us that seed transmission was unlikely. The seed was shipped to Japan, but the questions remained. What was the cause of dogwood canker? How was it transmitted?

Various cankers on dogwoods have undoubtedly been noted and discussed by growers for many years, but Gouin (1, 2) was the first to publish a description and illustrations of what is now called dogwood canker. Lambe (3) reported that nurserymen in Virginia noticed a trunk canker disease on dogwoods brought in from out-of-state. Lambe and Wills (4), using the term "dogwood canker," reported the isolation of several fungi from cankers, but found that none of these induced cankers following artificial inoculation. They also noted that neither plant pruning nor treatments with several fungicides were successful in preventing cankers. In further studies (5), they stated that more than 50% of white-bracted seedlings in a Virginia nursery were cankered, and that plants of the grafted pink-bracted cultivar 'Cherokee Chief' were less often cankered (*ca.* 10%) than those of the white-bracted 'Cherokee Princess' (41%). Their finding of significant differences in cankering among seedling lots produced by nurseries in 3 different States (4) may have contributed to the idea of seed transmission of the canker. However, the occurrence of cankers on cultivars propagated by grafting makes seed transmission less likely as a general mode of plant infection.

Materials and Methods

In January, 1983, we began our investigations by making numerous cross and longitudinal saw cuts through small and large cankers on 20 freshly-cut stem sections of flowering dogwood. The patterns of cell necrosis (Fig. 2) in the cankers suggested that the causal organism had a restricted mobility, both vertically and circumferentially. The fact that limited cell death occurred along different radii in different years meant either that new "infections" were occurring from outside each year or that the causal organism was capable of living in the tree (more than likely in the bark) and could then kill cambial tissue at various places in various years. There was no indication of annual wounds from the outside. The only organisms that we thought suited the observed facts were nematodes. Small segments of bark from cankers were placed in water in a petri dish and the following day two different nematodes were isolated.

Between January 1983 and July 1985, we attempted nematode isolation from 70 established cankers, 50 of which had been harvested as branch or trunk segments and 20 that were left on the plants. Bark samples from non-cankered areas were always taken as controls. Numbers of nematodes per unit area of bark were determined for each isolation.

This success led us to examine tissue from bark-disrupted areas (Fig. 3) on young sugar maple (*Acer saccharum* Marsh.) trunks that had been brought to our attention by W.N. Wandell, an Illinois nurseryman. Many cultivars of landscape trees are budded in Oregon and shipped East as 1-year whips. It was of extreme interest that, among several cultivars of sugar maple handled in this manner, a fairly regular, but low (up to 15%) percentage of only cv. 'Green Mountain' possessed these bark "tumors." Tress of this cultivar propagated in New Jersey did not exhibit any disruptive growth. Also, throughout 1983 and 1984, burls and tumors on a wide range of mature landscape trees were examined for nematodes. Generally, a total of about 50 cm^2 (8 in^2) of bark was removed, most often from the edge of the "cankered" area, with a hammer and chisel. Sub-samples were placed in water for nematode isolation. As with the dogwoods, bark samples of sugar maple and the other tree species from non-cankered areas were taken as controls.

The two nematodes isolated from dogwood cankers were tentatively identified as species of *Aphelenchoides* and *Panagrolaimus*. These identifications were confirmed by cooperating nematologists who considered the stylet-bearing nematode to be closely allied to *A. fragariae* (Ritzema Bos) Christie and the non-stylet species as *P. subelongatus* (Cobb) Thorne. Only *P. subelongatus* was isolated from sugar maple. Both nematodes were reared monoxenically on cultures of the fungus *Botrytis cinerea* Pers. ex Fr. growing on PDA (potato-dextrose agar).

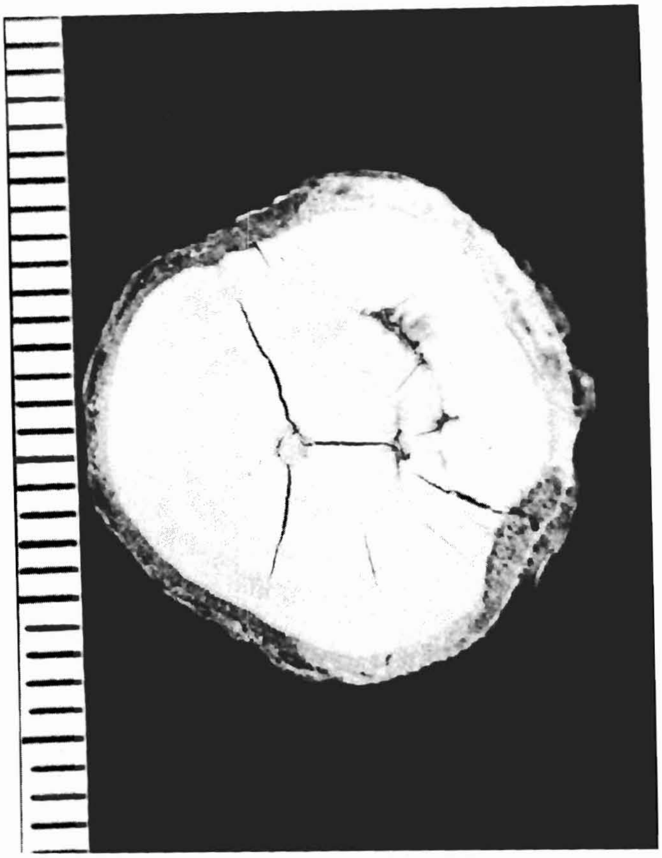
The first successful attempts to inoculate nematodes into young dogwood stems were made on June 2, 1983, with "success" being determined by reisolation of nematodes 50 days after inoculation. The inoculation technique consisted of (1) forming an inverted cone of parafilm around the stem; (2) sealing the cone to the stem with lanolin; (3) pouring a water suspension of nematodes into the cone, and (4) using a scalpel to make incisions through the bark below the water level. The cones were left on the stems until all the water had disappeared. Depending on the size of the stem inoculated, between 3 ml and 5 ml of the nematode suspension were used for each inoculation, with a nematode density of 100 to 150 per ml.

Between July 27 and August 15, 1983, 76 inoculations of *Aphelenchoides*, 17 of *Panagrolaimus*, and 24 of a mixture of the 2 nematodes were made into the 1982 or 1983 stem growth of *C. florida* growing outdoors in a mulched cold frame. Thirty-seven 3-year old seedlings and 12 plants representing seven cultivars were used as hosts. In addition to water controls, 10 inoculations were attempted without wounding the stem. The Chinese dogwood (*C. kousa* Hance) is considered to be "resistant" to dogwood canker, and 5 plants of this species were inoculated with nematodes, singly and in combination.

Attempts were also made to inoculate *Panagrolaimus* into stems of 3-year-old seedlings of sugar maple, Norway maple (*Acer platanoides* L.), red oak (*Quercus rubra* L.), white ash (*Fraxinus americana* L.), American linden (*Tilia americana* L.), and Chinese elm (*Ulmus parvifolia* Jacq.).

Although there was no evidence that the nematodes would be more viable in undifferentiated callus, we considered this possibility. In August, 1984, we made 57 inoculations on seven 5-year-old trees, four of which had natural cankers on the main trunk or on branches. Thirty-five inoculations were made into callus tissue that had developed following removal of a patch of bark from 1-year-old branches in June, 1983 or June, 1984. Twenty-two inoculations were made in the normal manner on previously uninjured stems.

Since it was suspected that the nematodes would be insect-vectored, numerous insects, mostly leafhoppers, found on the dogwoods were examined for external or internal ne-



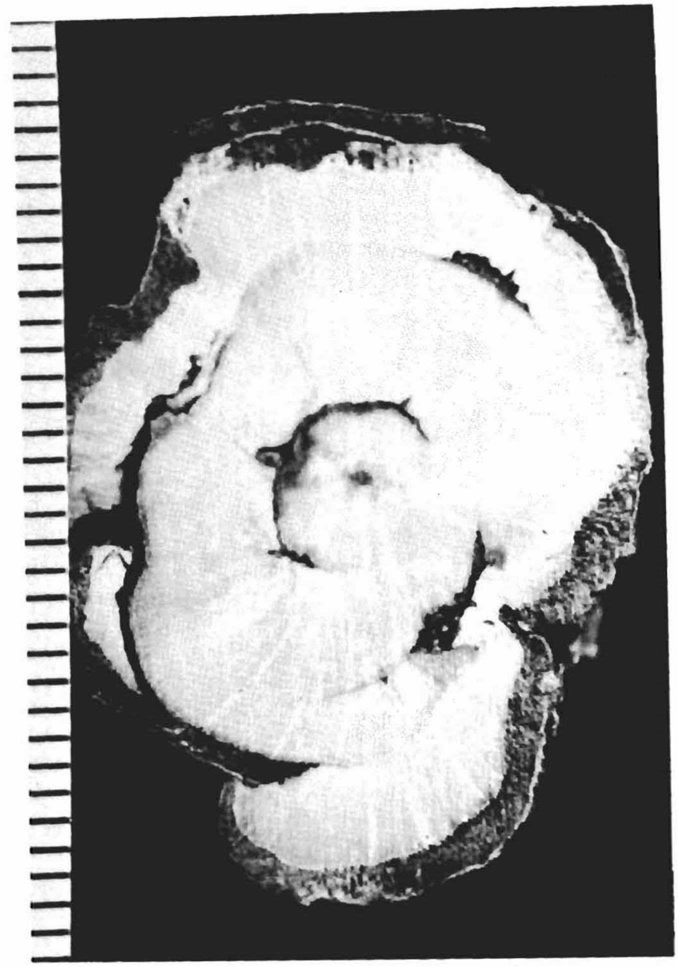


Fig. 2. **Cross-sections through cankered areas of flowering dogwood stems, showing cell necrosis (dark regions in xylem) coincident with annual rings and with varying location and intensity/from year to year. Light-colored bark is alive and dark bark is dead. Each division on scale is 1 mm.**

matodes. However, we did not observe any insect or other animal actually making feeding or ovipositing penetrations into the bark.

Attempts to re-isolate nematodes from inoculated bark areas were made at various times from May to September, 1984 for the first series of inoculations and from June through July, 1985 for the second series.

Results and Discussion

Both nematode species were isolated from 80% of the 70 natural cankers on *C. florida* that were examined, and in no case was only a single species isolated. The maximum number of nematodes per cm^2 (.16 in^2) of cankered bark was 12, and the average was approximately three. No nematodes were found in the bark of any non-cankered area of these young dogwoods.

The only nematode isolated from the tumorous growths on 'Green Mountain' sugar maple was *P. subelongatus*. Only three freshly-cut trunks and two living trees were available for observation but repeated sampling of those two trees until the total area of bark disruption was removed did not reveal any other nematode. Again, the number of nematodes was low, and they were only found in bark within or immediately adjacent to the disrupted area.

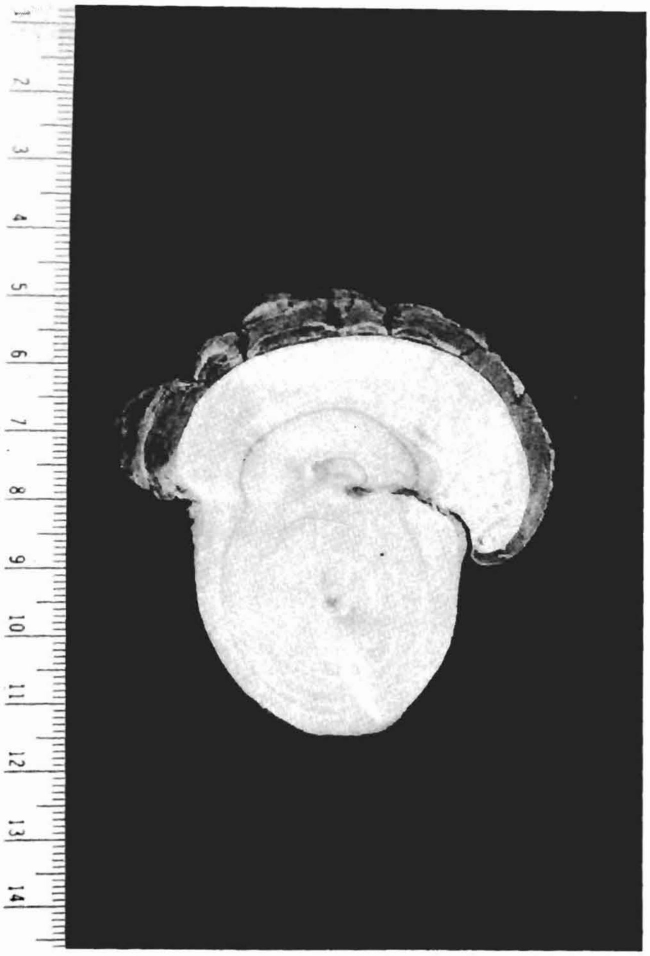


Fig. 3. **Cross-section through tumor on sugar maple 'Green Mountain.' Note absence of severe cell necrosis in xylem and accentuated wood development and very thick bark in tumored area. Scale is in centimeters.**

Fig. 4. **Irregular burl on black locust. Scale is 15 cm (6 in) long.**

We were able to isolate both nematode species from "burls" on large landscape trees of red maple (*Acer rubrum* L.), Higan cherry (*Prunus subhirtella* Miq.), red oak (*Quercus rubra* L.), black locust (*Robinia pseudoacacia* L.) (Fig. 4), and Siberian elm (*Ulmus pumila* L.) (Fig. 5). The numbers of nematodes were low, but we did not attempt to quantify their density. In red maple and Siberian elm, there were many "burled" trees in the same planting, suggesting that these abnormal growths may have originated during the time they were in the nursery.

The nematode inoculations made in dogwood in 1983 were harvested and checked for nematodes at various times over a prolonged period from May to August, 1984. The results were totally negative and we were not able to isolate nematodes from any inoculated dogwood. Likewise, no nematodes could be isolated from any of the other tree species that were inoculated. If nothing else, these results should confirm the non-existence of nematodes in healthy bark of these young trees.

The 1984 inoculations made into callused bark of dogwoods yielded no nematodes when examined in 1985. However, the bark adjacent to several inoculation sites did appear roughened, and dissection of six stems revealed the early stages of the cell necrosis patterns that were characteristic of more mature cankers (see Fig. 2). In addition, we could not isolate any nematodes from the surface or entrails of any of the insects we examined.

With all of this negative evidence, there is still a possibility that nematodes are the cause of dogwood canker. Our failure to find nematodes in every canker could be explained by the small numbers of nematodes involved coupled with a continual reduction in the amount of living bark suitable for their development. Furthermore, it is likely that low winter temperatures play a role in limiting the development of cankers. The fact that dogwood cankers are not found on nursery stock grown in northern nurseries lends some credibility to this hypothesis. The critical time period may be the first winter following infestation. If the nematodes survive that first winter, they are likely to be better protected by thicker bark during succeeding winters. The lowest temperature recorded at the Arboretum during the winter after our 1983 inoculations was −22°C (−4°F). We were still able to find nematodes in previously established cankers in 1984 but could not examine enough to determine whether a significant reduction in numbers had occurred. It would be interesting to follow the course of canker development in young nursery plants following the disastrous winter of 1983–84 in Tennessee.

Some of our problems in re-isolating nematodes the year after inoculation could be the result of inefficient inoculation technique. On the other hand, we are not certain as to the degree of success achieved by "natural" nematode vectors.

Fig. 5. Regular burls on Siberian elm. All trees in this landscape planting were burled and all had a common nursery origin.

We postulate that nematodes may be responsible for various growth disruptions in woody stems by (1) killing cambial cells and (2) producing hormone-like substances that stimulate cambial activity or cell enlargement. The necrotic zones at the borders of the annual rings in dogwood cankers (Fig. 2) are evidence of cell death. The ''lopsided'' growth pattern shown in the cross-section through 'Green Mountain' sugar maple (Fig. 3) may be evidence of an hormonal stimulus only, keeping in mind that only the non-stylet *Panagrolaimus* was associated with these growths. Cross-sections through burls on black locust and Siberian elm revealed erratic growth patterns similar to those of dogwood.

The degree of stem involvement in the various abnormal cankers, tumors, or burls must depend on the size or age of the plant when infested and its subsequent growth rate. If, as has been determined, dogwood stems become infested during their first or second year of growth, their small size and relatively slow growth rate usually allows the entire stem circumference to become part of the canker. If the infestations occur later in life, as evidenced in the mature-tree burls, the ensuing bark disruptions are more ''one-sided.''

As a final hypothesis, it is our opinion that neither dogwoods nor any of the other trees harboring nematodes in disrupted bark are ''normal'' hosts for the nematodes nor perhaps even for their vectors. The rarity of such burls and cankers in the natural forest may be taken as evidence on this point. Certainly, it does not appear that infested dogwoods or other trees could be classified as ''carriers'' and pose any threat to non-infested trees. Rather, we suspect that the introduction of nematodes into woody stems is an accidental and incidental occurrence, the probability of which is enhanced under ''unnatural'' growing conditions in nurseries located in areas with normally mild winters.

Significance to the Nursery Industry

This study is the first to demonstrate a rather constant association of particular infectious organisms with the problem known as dogwood canker. Dogwood cankers are, for the most part, initiated under normal production procedures in southern nurseries. While we, and others, continue to search for nematode vectors and work toward establishing a direct cause-and-effect relationship between nematodes and cankers, nurserymen might be able to reduce canker incidence by any cultural or other practice that will provide less favorable habitats for insects and nematodes.

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