Butternut Canker: Development on Individual Trees and Increase Within a Plantation

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ABSTRACT

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The incidence of butternut canker, incited by *Sirococcus clavigignenti-juglandacearum*, increased exponentially from 5% in 1978 to 76% in 1983 within an isolated butternut (*Juglans cinerea*) plantation. On individual trees, cankers developed first on branches in the lower crown. Branch mortality and sporulation by the fungus followed. Trunk cankers developed 1–3 yr after initial branch mortality. Trees with tops killed by coalescing basal cankers did not resprout at the root collar. The fungus continued to colonize and sporulate on dead trees for 20 mo. Felling or treating cankered trees with a silvicide failed to prevent fungal colonization and sporulation.

Butternut canker, incited by *Sirococcus clavigignenti-juglandacearum* Nair, Kostichka, & Kuntz, is lethal to butternut (*Juglans cinerea* L.). Diseased trees show multiple cankers on branches, trunks, and buttress roots. Cankers may quickly girdle twigs or become perennial on larger limbs and the trunks (8). Perennial trunk cankers are elliptical, vertically oriented, and may be partially covered by shredded bark (8).

Stromata are produced in the periderm of dead branches and trunks of butternut. Stromatal columns (2–3 mm long), previously termed hyphal pegs (5), rupture the outer bark, exposing stromata and pycnidia. We believe the term stromatal column is more appropriate than hyphal peg because the column originates in, and is similar in structure to, the stroma. Stromatal columns also bear pycnidia (4). The term hyphal peg has been used previously to describe interwoven hyphae projecting from the trama of certain basidiocarps (2). Therefore, its use in describing stromatal characteristics in *S. clavigignenti-juglandacearum* may lead to confusion.

Butternut canker has caused widespread mortality of butternut in Wisconsin (8) and now is reported throughout much of the natural range of the species in midwestern and eastern United States (1,8). Elimination of butternut in certain areas of the country may result in the tree becoming a threatened species (6). This study reports on the development of butternut canker on individual trees, the progression of an epidemic in a plantation, and the survival of *S. clavigignenti-juglandacearum* in dead trees.

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MATERIALS AND METHODS

Study plot. Disease development on individual trees and the progression of an epidemic with time were studied in an isolated butternut plantation established in 1972 at the University of Wisconsin Experimental Farm near Arlington. The plantation, containing 994 butternut trees, was about 400 m long in a north-south direction and 24 m wide. Trees were planted in five rows spaced 4 m apart (except for an 8-m gap between rows 3 and 4). Spacing between trees in a row varied from 1 to 5 m. All butternut trees in the plantation were canker-free before 1976. The nearest cankered butternut trees in natural stands outside the plantation were 6 km to the east.

Inoculations. In April 1980, 30 symptomless butternut trees were selected from the northern edge of the plantation. Lower branches that had been killed by shading were removed. At about 2-wk intervals from 23 April to 7 August, a different branch (5–15 mm diameter) on each of 10 trees was inoculated at three wounded leaf scars with 0.5 ml of spore suspension (10^6 conidia per milliliter). The wounds were incisions 5 mm long and 2–3 mm deep made with a scalpel. The culture of *S. clavigignenti-juglandacearum* was a mass isolate obtained from a cankered tree in the University of Wisconsin Arboretum. Spore suspensions were prepared from 10- to 20-day-old cultures grown on 2% potato-dextrose agar (PDA). After inoculation, leaf scars were covered loosely with aluminum foil to prevent rapid desiccation. The foil was removed after 1 wk. Because all branches on some of the smaller trees had been inoculated by 7 August, branches on 10 additional trees were inoculated similarly at about 2-wk intervals from 18 August to 10 October. At each inoculation date, branches on 10 other trees were wounded at leaf scars and treated with 0.5 ml of sterile distilled water. The experiment was repeated in 1981 on 10 trees.

Disease progression. Between 1976 and spring 1978, 50 trees in the northwestern corner of the plantation were inoculated on branches and trunks with *S. clavigignenti-juglandacearum*. Inoculations were made with a knife by cutting through the bark to the sapwood and inserting small pieces of PDA containing mycelium and conidia of the fungus. By May 1978, all inoculated trees had developed branch and trunk cankers. Therefore, the initial disease incidence was 5% (50 of the 994 trees). Branches of 20 additional trees in 1980 and 10 more trees in 1981 were inoculated with spore suspensions of the fungus.

At least once a year from May 1980 to May 1983, each butternut tree in the plantation was inspected for branch and trunk cankers or for dead branches bearing stromatal columns of the fungus. The rates of increase of incidence of diseased trees and of trees with trunk cankers were analyzed by the exponential model:

$$r = (\log_e x_t - \log_e x_o)/\Delta t,$$

where r = logarithmic infection rate ($\log_e$ units/yr), x_t = incidence in year t, and x_o = initial incidence (11).

Colonization of dead butternut trees. Experiments were conducted to determine whether *S. clavigignenti-juglandacearum* continued to colonize and sporulate on girdled, poisoned, or felled butternut trees. In February and September 1980, 12 trees with multiple cankers were felled in the University of Wisconsin Arboretum at Madison. The diameter at breast height (dbh) and height of each tree, as well as the number of visible cankers and their locations, were recorded. At least once a month from May through October, Vaseline-coated microscope slides (one or two per tree) were mounted horizontal to the ground and flush against loosening trunk bark bearing stromatal columns and pycnidia of the fungus. Slides were exposed for 4- to 7-day intervals in which there was rainfall. A spore deposition index (SDI) was determined for each slide, similar to that described by Johnson and Kuntz (3). On each slide, 20 random light-microscopic

fields (×430) were viewed and scored as follows: 0 = no spore deposition, 1 = 1–10 conidia, 2 = 11–100 conidia, and 3 = more than 100 conidia.

Felled trees were examined for stromatal columns and cirri. When these were not found, samples of the bark and wood were brought to the laboratory for microscopic examination and isolation on acidified PDA.

A similar experiment was initiated on 23 September 1981 in a small wood lot about 15 km north of Spring Green, WI. Twelve trees with multiple branch and trunk cankers and ranging in height from 6 to 12 m and in dbh from 16 to 28 cm, were selected from the open hardwood stand. Four trees were felled and left on the forest floor under the canopy to hasten colonization of bark and wood by saprophytic decay organisms. Four trees were girdled with an axe through the bark and into the sapwood at a height of 0.25 m. Four other trees were girdled and treated with the silvicide monosodium acid methanearsonate (Silvisar 550, 48% a.i.) by pouring about 10–15 ml of the chemical into the wound. Girdling or girdling and poisoning were intended to rapidly dry and loosen the bark, thus preventing extensive colonization and sporulation by *S. clavigignenti-juglandacearum*. All trees in the experiment were examined in May and September 1982 for stromatal column production and sporulation by the fungus.

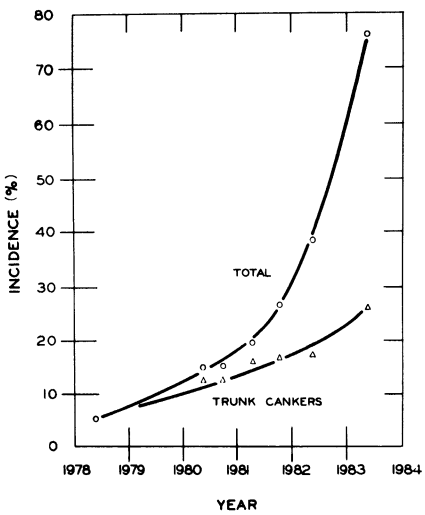

Fig. 1. Disease progression in an isolated butternut plantation. Incidence in 1978 represents 50 trees that were inoculated on branches and the trunk with mycelium and conidia of *Sirococcus clavigignenti-juglandacearum*. Total incidence includes trees with branch and trunk cankers or branch cankers only.

RESULTS

Inoculations. In 1980, inoculations at every date incited branch cankers (Table 1). Initial lesion development on individual branches was variable with time, even for inoculations on the same date. Subsequently, two types of canker development were observed. In one type, a sunken, elliptical canker developed around the point of inoculation. Cankers expanded slowly in the bark, and branches remained alive for a year or more. In the other type, the fungus rapidly colonized bark tissue and no distinct canker was formed. Some branches were girdled and killed within 1 mo of inoculation; on these, the fungus developed stromatal columns. Branches rapidly colonized by the fungus usually were located in the lower portion of the tree crown. Results of inoculations in 1981 were similar.

Of all branches inoculated in 1980, 47% were cankered by April 1981 (Table 1). By November 1981, 68% of the inoculated branches were cankered, and of these, 77% bore stromatal columns. Thus, some cankers did not form until the second growing season after inoculation. Wounds on branches treated with distilled water were closed tightly by marginal callus after 1 yr.

By October 1982, six of the 20 trees inoculated in 1980 had developed trunk cankers. On four of the six trees, cankers were located at the root collar region. Two other trees developed trunk cankers at the junction of the inoculated branch and the main stem. By May 1983, 10 trees had one or more trunk cankers. None of the trees inoculated in 1981 had developed trunk cankers.

Disease progression. In the plantation, disease incidence, which included trees with branch and trunk cankers or trees with branch cankers only, increased from 5% in 1978 to 76% by May 1983, with a logarithmic infection rate of 0.52 $\log_e$ units per year ($r^2 = 0.98$) (Fig. 1). Some of the increase in incidence resulted from artificial inoculations in 1980 and 1981 (3%), which may have influenced the infection rate. The incidence of trees with trunk cankers also increased with time at an infection rate of 0.31 $\log_e$ units per year ($r^2 = 0.94$); by May 1983, 26% of all trees in the plantation had one or more trunk cankers. Branch cankers and branch mortality preceded the formation of trunk cankers by 1–3 yr. Trees that became infected naturally first developed irregular or diffuse cankers on branches in the lower crown. Branch mortality, stromatal column production, and sporulation of the fungus followed. Discrete, elliptical lesions surrounded by callus were found rarely on the branches during the early stages of the disease. Of the trees with branch mortality in 1980, 67% developed one or more trunk cankers by May 1983.

Dead branches or branch stubs were associated with 71% of all trunk cankers in the plantation. Most trunk cankers were elliptical or irregular in shape and were bordered by callus. However, some stem cankers were diffuse, ie, they had not formed callus. In some cases, diffuse cankers extended 50 cm or more along the trunk. By May 1983, 19 of the naturally infected trees had been girdled and killed by multiple trunk cankers. An additional 52 naturally infected trees had about 75% of the trunk circumference killed by coalescing cankers.

Of the trees with trunk cankers, 72% had one or more basal cankers within 20 cm of the soil line. The root collars of six trees with basal cankers were excavated. In all cases, cankers originated above the soil line and had advanced into the roots. On one tree, discoloration of the lateral root extended to a depth of 25 cm in the soil (Fig. 2). *S. clavigignenti-juglandacearum* was isolated from necrotic roots on all trees sampled. Stromatal columns were not found on necrotic root tissue.

Table 1. Canker formation and stromatal column production on butternut trees after inoculations of wounded leaf scars with *Sirococcus clavigignenti-juglandacearum* at different times of the year in 1980

	Inspection date					
	November 1980		April 1981		October 1981	
Inoculation date	Canker[a] (%)	Stromatal columns[b] (%)	Canker (%)	Stromatal columns (%)	Canker (%)	Stromatal columns (%)
23 April	50	40	50	40	70	57
3 May	80	63	80	75	90	67
14 May	30	100	30	100	60	83
6 June	40	25	40	50	60	83
20 June	70	29	80	38	80	75
12 July	60	83	60	83	80	75
24 July	40	50	40	100	60	83
8 August	20	50	20	100	30	67
18 August	30	100	50	60	50	100
9 September	10	100	40	25	70	71
22 September	0	0	60	0	80	88
10 October	0	0	10	0	80	75

[a] Percentage of branches developing cankers for each inoculation date. Branches on 10 trees from 23 April to 7 August and 10 trees from 18 August to 10 October were cut at leaf scars with a scalpel, producing a wound 5 mm long by 2 mm deep. Each wound was inoculated with 0.5 ml (10^6 conidia/ml) of spore suspension.

[b] Percentage of cankered branches bearing stromatal columns and pycnidia of the fungus.

Colonization of dead trees. In April 1979, it was noted the *S. clavigignenti-juglandacearum* continued to sporulate profusely on butternut trees that had been killed by the disease the preceding year. The fungus colonized large areas of the trunk and branches beyond canker margins, producing stromatal columns that ruptured the outer bark (Fig. 3). The fungus sporulated on dead, standing butternut trees throughout the summer.

In 1980, 12 trees with multiple cankers were felled and left on the forest floor. The numbers of externally visible cankers and their locations on 10 of the trees are presented in Table 2. All trees had multiple cankers of the trunk, root collar, and buttress roots. One tree had 51 cankers on the trunk alone. Cankers were distributed over the entire length of the stem, but the mean number of cankers per stem section decreased with increasing height on the trunk. Some cankers were associated with branch stubs; others were located in areas free of branches. Cankers were not aligned on one side of the trunk. All felled trees had extensive branch mortality resulting from infection in the lower crown; the upper crown generally had few cankers or dead branches.

Cankered butternut trees felled in February and September 1980 became colonized extensively within 8 mo. Six trees felled in September 1980 had a mean SDI of 2.8 after 8 mo; sporulation decreased to a mean SDI of 1.7 after 20 mo. Thereafter, trunk bark had sloughed from all felled trees and the wood was colonized by wood-decaying fungi. No sporulation of *S. clavigignenti-juglandacearum* was detected and the fungus could not be isolated from bark and wood samples 2 yr after felling.

In September 1981, cankered trees on a xeric, upland site were either felled, girdled, or poisoned. Felled trees lying on the forest floor were colonized by *S. clavigignenti-juglandacearum* within 8 mo. Girdling did not immediately kill the trees and it did not prevent fungal sporulation. All girdled trees had an SDI of 3 after 8 mo. Trees that were girdled and treated with silvicide were killed by late October, but the treatment failed to loosen bark. By May 1982, all trees treated with the silvicide had stromatal columns and an SDI of 3.

DISCUSSION

Results indicate a characteristic pattern of disease development on individual trees. Branch cankers or dead branches bearing stromatal columns usually are found first in the lower crown of the tree. Infection of lower branches may result from a microenvironment favorable for conidial germination during and after rainfall. Conidia of *S. clavigignenti-juglandacearum* require at least 16 hr of dew at 20 C for germination on bark of butternut seedlings (9). Lower branches tend to retain moisture necessary for spore germination for longer periods than branches in the upper crown. In addition, the vigor of some lower branches is reduced by partial shading, which may make such branches susceptible to rapid colonization by the fungus. Such a reaction might explain the formation of diffuse cankers and the rapid death of some lower branches. However, infection and colonization by the fungus is not restricted to physiologically stressed tissue because branch and trunk cankers also are found on vigorously growing trees. The fungus sporulates profusely on necrotic tissue of the lower branches, producing inoculum for further infection. The development of additional branch cankers and, ultimately, branch mortality progresses from the lower portion of the crown upward.

Trunk cankers commonly are associated with dead branches or branch stubs, but they also may occur at wounds or natural openings as a result of infection by conidia washed down the trunk from dead branches during rainfall (9). The formation of trunk cankers in the plantation did not occur until 1–3 yr after branch mortality. This indicates that pruning the lower branches might reduce initial infections and the number of subsequent trunk cankers. Nevertheless, multiple trunk cankers on large-diameter butternut trees with 5 m or more of clear stem indicate that removal of diseased branches will not eliminate the formation of trunk cankers.

An unusual feature of the disease is the formation of basal stem and root cankers. Cankers advance below the soil line and even into lateral roots. Girdling of the main stem commonly occurs at the root collar; trees killed in this manner do not regenerate by sprouts. Excavation of trees shows that the fungus is capable of colonizing root tissue, but the distance of colonization and the longevity of the fungus in roots are not known.

At present, there are no effective

Fig. 2. Basal trunk canker associated with a branch stub on a butternut tree. The canker has progressed into the roots.

Fig. 3. Stromatal columns of *Sirococcus clavigignenti-juglandacearum* that have ruptured the outer bark and exposed sporulating pycnidia.

Table 2. Numbers and distributions of visible cankers on 10 naturally infected butternut trees felled in February and September 1980

Canker locations	No. of cankers[a] Mean	Range
Trunk		
0.0–1.2[b]	7.4	3–13
1.3–2.4	7.1	2–13
2.5–3.6	5.5	2–10
3.7–4.8	4.5	1–8
4.9–6.0	3.7	0–9
>6.0	2.5	0–10
Total	30.7	13–51
Branch	4.2[c]	0–10

[a] Mean number and range of externally visible cankers on 10 trees, ranging in height from 9.0 to 12.1 m and in diameter at breast height from 7.6 to 15.2 cm.

[b] Distance (m) from the root collar toward the crown.

[c] Mean number of branch cankers does not include numerous dead branches with stromatal columns.

control measures for butternut canker. Inoculations have shown that butternut tissue is susceptible throughout the growing season. The fungus can survive saprophytically in and sporulate on dead butternut trees for at least 20 mo. Conidia are dispersed in water droplets or aerosols during rain, and can remain viable in air for at least 8 hr (10). This could allow dispersal of viable conidia for distances of 1 km or more, even at relatively low wind speeds. The disease is prevalent throughout the range of butternut and increases exponentially with time in a population. For these reasons, it is doubtful that butternut canker can be controlled by sanitation measures. Butternut trees that appear healthy have been found growing among cankered trees (6). Whether such trees escaped disease or resisted fungal attack is uncertain. Canker-free trees or trees with small, well-callused cankers should be tested for possible resistance. Japanese walnut (*J. ailantifolia* Carriere) is reported resistant to fungal attack (7), and might be useful in future breeding programs with butternut.

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