Control of *Nectria cinnabarina* Cankers on Honey Locust

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ABSTRACT

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Four cultivars of thornless honey locust were inoculated with *Nectria cinnabarina*, a canker-causing pathogen of honey locust in Minnesota, to determine their relative susceptibility. The cultivars Sunburst, Skyline, and Imperial were about equal in susceptibility to infection, and Thornless was more resistant. Concentrations of benomyl exceeding 1 μg/ml totally inhibited growth of *N. cinnabarina* in vitro. Five-year-old honey locust trees were wounded and then inoculated with *N. cinnabarina* after various intervals. Two concentrations of benomyl were tested for efficacy in preventing canker formation. Wounds inoculated 2 hr or 1 day after wounding developed cankers. Wounds inoculated 1 wk or more after wounding were not significantly larger ($P > 0.05$) than the uninoculated wounds. Wounds sprayed with a solution of 10 μg/ml benomyl and subsequently inoculated with *N. cinnabarina* were not significantly different ($P > 0.05$) from unsprayed wounds. However, wounds sprayed with a concentration of 100 μg/ml benomyl and inoculated 1 day after wounding were significantly smaller ($P < 0.05$) than unsprayed, inoculated wounds.

Nectria cinnabarina (Tode:Fr.) Fr. is a serious canker-causing pathogen of thornless honey locust (*Gleditsia triacanthos* L. var. *inermis* Willd.) in Minnesota (2). Control procedures suggested for this disease include the following. 1) Avoid excessive pruning wounds or other injuries; 2) do not prune during wet weather, when inoculum is abundant; and 3) avoid stress because vigorously growing trees are less susceptible to disease (1,2,9). Because these control procedures alone do not appear adequate to control this disease, additional methods are needed.

N. cinnabarina has a global distribution (4), with an extremely broad host range (9). This fungus is thought to occur mainly as a saprophyte and occasionally as a weak parasite (5). In Holland, successful control of cankers caused by *N. cinnabarina* on elm was achieved through selection of resistant clones (7). Because *N. cinnabarina* is so widely distributed, selecting for resistant specimens may be the most successful and reliable method for control.

Wounds are required for infection by *N. cinnabarina* to occur (2); therefore, it may be possible to reduce the incidence of cankers by using fungicides or other compounds to protect wounds from infection. The in vitro sensitivity of fungi to benomyl is generally correlated with taxonomic status (3). Many Ascomycetes, especially those with phialidic anamorphs, are very sensitive to benomyl (3). Bollen and Fuchs (3) state that in vivo activity of benomyl can be predicted with great certainty from data on in vitro inhibition of fungal growth.

This study was undertaken to determine 1) relative susceptibility of four commonly planted cultivars of honey locust in Minnesota to *N. cinnabarina*, 2) in vitro sensitivity of *N. cinnabarina* to benomyl, 3) efficacy of benomyl applied to wounds on field-grown honey locust trees in preventing colonization by *N. cinnabarina*, and 4) length of time wounds remain susceptible to colonization by *N. cinnabarina*.

MATERIALS AND METHODS

In May 1982, 20 bare-root honey locust trees of each of four cultivars (Table 1) were planted in a nursery on the St. Paul campus of the University of Minnesota. The trees were 2-yr-old, 1.5-m whips. Ten trees of each cultivar received two wounds per tree on 28 June or 3 September 1982 (Fig. 1). Wounds 1 cm long and 0.5 cm wide were made with a sterile scalpel through the outer bark and phloem. An isolate of *N. cinnabarina* (IMI 262937) from the margin of an active canker on a honey locust tree was grown on sterile oats for 4 wk prior to inoculation. A single colonized oat kernel was placed in one wound per tree. A sterile oat kernel was placed in the second wound. Wounds were covered with Parafilm. Eleven weeks after inoculation, the trees were cut and the length and width of the dead cambium were measured by removing the outer bark and phloem from around the wound with a scalpel. Isolations were made to recover *N. cinnabarina*, using techniques described previously (1).

In vitro sensitivity of *N. cinnabarina* was determined for various concentrations of benomyl (Table 2). A 100-μg/ml stock solution was prepared by suspending 0.02 g benomyl (Benlate 50W, 50% a.i.) in 100 ml of sterile distilled water. This stock solution was then added in appropriate quantities to Difco potato-dextrose agar (PDA) to give the desired concentrations of benomyl. Disks 1 cm in diameter were removed with a sterile cork borer from the margins of 1-wk-old cultures of *N. cinnabarina* and placed in the centers of petri dishes containing various concentrations of benomyl-amended PDA. Diameter measurements of colony growth, along two axes at right angles to

Table 1. Comparison of lesion length on four cultivars of honey locust inoculated with *Nectria cinnabarina* at two different times

Cultivar	Inoculation date	Lesion length (cm)[x]	
		Inoculated[y]	Uninoculated[z]
Sunburst	28 June	4.22 a	1.15*
	3 September	2.60 bc	1.61
Skyline	28 June	3.91 a	1.01*
	3 September	2.19 c	1.32
Imperial	28 June	3.47 ab	1.40*
	3 September	2.13 c	1.28
Thornless	28 June	1.99 c	1.41
	3 September	1.83 c	1.10

[x] Average value of 10 replicates.

[y] Average values followed by the same letter in a column were not significantly different ($P = 0.05$) using Bonferroni's method of multiple comparisons (11) (BSD = 1.26 cm).

[z] An asterisk denotes the average values within a row were significantly different ($P = 0.05$) using Bonferroni's method of multiple comparisons (11) (BSD = 1.26 cm).

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each other, were taken every 48 hr until the fungus had completely covered the plates not containing benomyl. These two measurements were used to determine the average diameter growth of the culture. During this study, the cultures were maintained in a dark incubator at 25 C.

The efficacy of benomyl in inhibiting infection of wounds by *N. cinnabarina* was tested on field-grown honey locust trees. On 29 June 1982, 60 5-yr-old honey locusts (cultivar Skyline) received four wounds on the main stem. Wounds 4 cm long and 2.8 cm wide were made with a metal template cutter (Fig. 2). The four wounds were spiraled up the tree 25 cm apart. Immediately after wounding, two of the four wounds were sprayed to runoff with either a 10- or 100-μg/ml aqueous suspension of benomyl. The remaining wounds were sprayed with sterile distilled water. Three wounds on each tree were inoculated at one of six different times (Table 3) after wounding. Ten replicates were used for each treatment. The inoculum was grown on sterile oats for 4 wk before inoculation. About 25 colonized oat kernels on a moist, sterile gauze pad (6 × 8 cm) were placed on the wound (Fig. 3). Two wounds sprayed with benomyl and one sprayed with sterile distilled water on each tree were inoculated with *N. cinnabarina*. The remaining wound received sterile oats on a gauze pad. All wounds were wrapped with Parafilm and plastic flagging to keep the gauze in place and retain moisture. Fourteen weeks after each inoculation, the trees were cut and isolations made to recover *N. cinnabarina*, using techniques described previously (1). The length and width of the dead cambium were measured after removing the outer bark and phloem from around the wound.

Table 2. Percent inhibition of the diameter of *Nectria cinnabarina* colonies grown 4 days at 25 C on potato-dextrose agar amended with various concentrations of benomyl

Concentration of benomyl (μg/ml)	Average colony growth (cm)[y]	Inhibition (%)
0.0	4.75 a[z]	0
0.1	3.61 b	24
0.5	3.33 b	30
0.8	1.61 c	66
1.0	0.80 d	81
3.0	0.00 e	100
5.0	0.00 e	100

[y] Each number represents the average of six replicates.
[z] Means in a column followed by the same letter did not differ significantly ($P = 0.01$) using Tukey's method of multiple comparisons (HSD = 0.47 cm).

RESULTS

The four cultivars of honey locust differed in susceptibility to *N. cinnabarina* when inoculated in early summer. Cankers that developed during the 11 wk after inoculation on Thornless were not significantly longer ($P > 0.05$) than the uninoculated wounds on this cultivar (Table 1). Inoculated wounds on Thornless were significantly smaller ($P < 0.05$) than those on the other three cultivars. Sunburst trees developed the largest cankers during the 11 wk after inoculation, averaging 4.22 cm long (Table 1). Skyline and Imperial trees developed cankers slightly smaller but not significantly different ($P > 0.05$) from the cankers on Sunburst. Cankers that developed as a result of inoculations conducted on 28 June 1982 were significantly longer ($P < 0.05$) than the uninoculated wounds on the three susceptible cultivars.

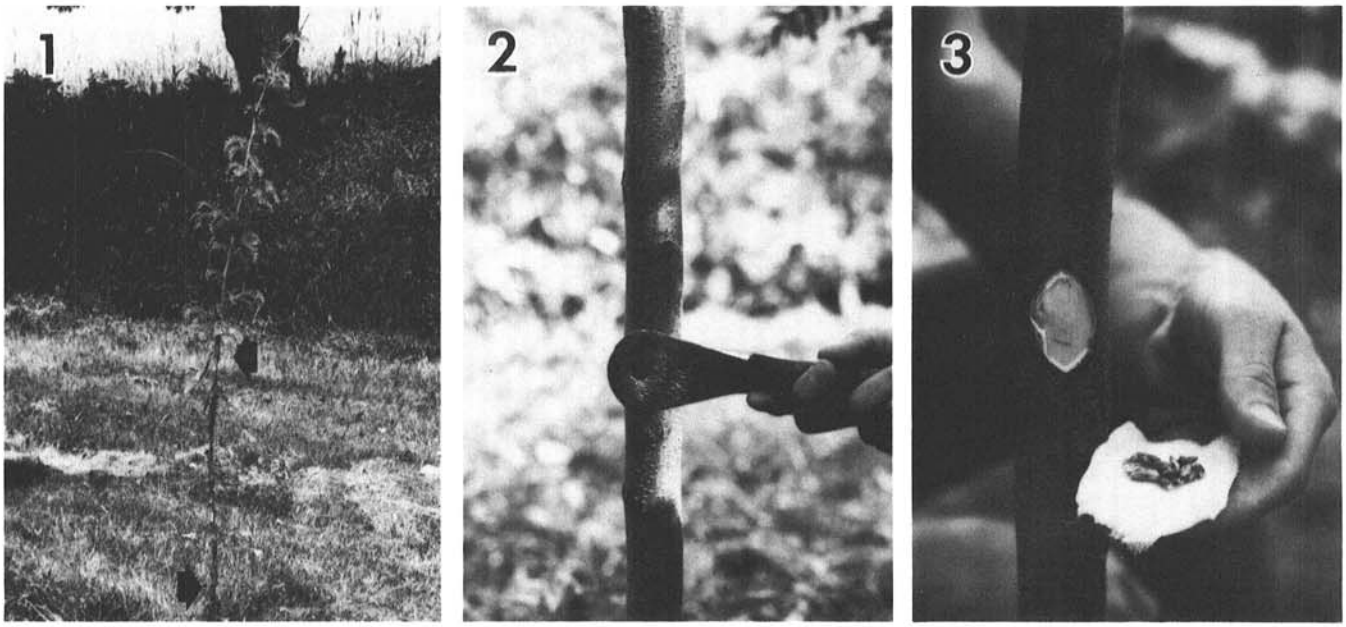
Sunburst, Skyline, and Imperial, which were susceptible to *N. cinnabarina* when inoculated on the 28 June 1982, did not develop cankers that were significantly larger ($P > 0.05$) than uninoculated wounds when inoculated on 3 September. Both inoculated and uninoculated wounds on Thornless treated in late summer were about the same size as those treated in early summer.

N. cinnabarina was recovered from

Table 3. Average lesion length (cm) of wounds treated or not treated with benomyl on honey locust trees inoculated with *Nectria cinnabarina* at various times after treatment[x]

	Benomyl concentration (μg/ml)	Delay between wounding and inoculation[y,z]					
		2 Hr	1 Day	1 Wk	2 Wk	4 Wk	8 Wk
Inoculated	0	6.01 a	6.20 a	5.18 a	4.31 a	4.39 a	4.42 a
	10	6.23 a	5.99 ab	4.85 a	4.52 a	4.31 a	4.45 a
	100	5.47 a	5.48 bc	4.62 a	4.56 a	4.29 a	4.35 a
Uninoculated		4.23 b	4.85 c	4.54 a	4.51 a	4.47 a	4.39 a

[x] All trees were harvested 14 wk after inoculation.
[y] Average value of 10 replicates.
[z] Values in a column followed by the same letter were not significantly different ($P = 0.01$) using Bonferroni's method of multiple comparisons (11) (BSD = 0.70 cm.).



Figs. 1–3. **(1)** Two-year-old Sunburst honey locust inoculated with *Nectria cinnabarina*, showing position of the wounds (arrows).**(2)** Five-year-old honey locust being wounded with a template wounding tool. **(3)** Oat kernels with *Nectria cinnabarina* placed on a sterile gauze pad being applied to a wound on a 5-yr-old honey locust tree 2 hr after wounding.

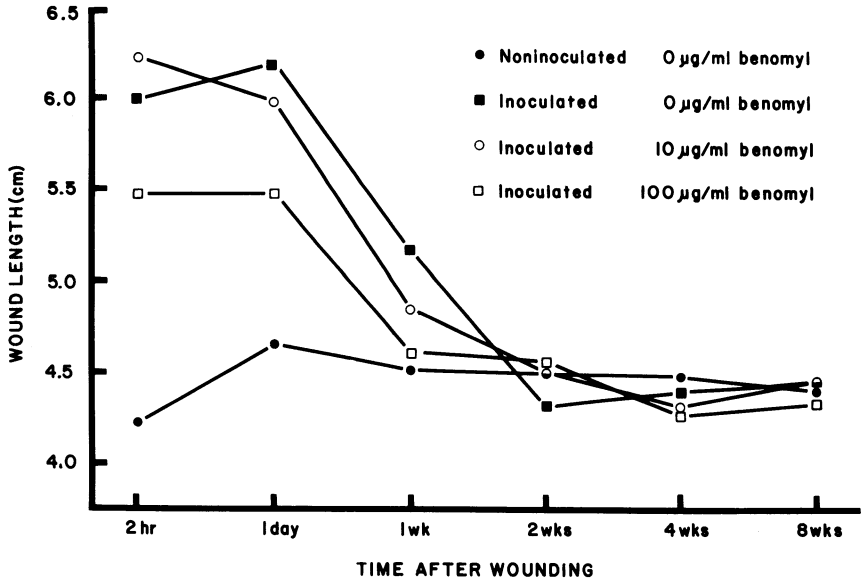


Fig. 4. Comparison of average wound length for four wound treatments at various inoculation times after wounding.

97% of the wounds inoculated on 28 June and 3 September. Nearly all wounds inoculated in early summer had formed callus tissue around the cankers before harvest. In contrast, none of the trees inoculated in late summer had formed callus during the 11 wk between inoculation and harvest.

Low concentrations of benomyl inhibited growth of *N. cinnabarina* in vitro. Colony size was reduced 24% after 4 days at a concentration of 0.1 µg/ml of benomyl, and growth was totally inhibited by concentrations exceeding 1 µg/ml (Table 2).

Wounds inoculated with *N. cinnabarina* 1 day or less after wounding were significantly larger ($P < 0.05$) than the uninoculated wounds for the same time period (Table 3, Fig. 4). Wounds inoculated 1 wk or more after wounding were not significantly different ($P > 0.05$) from uninoculated wounds treated at the same time (Table 3). There was a highly significant interaction ($P < 0.001$) between time of inoculation after wounding and wound treatments. Wounds sprayed with 10 µg/ml benomyl and inoculated with *N. cinnabarina* for all six inoculation times after wounding were not significantly smaller ($P > 0.05$) than the unsprayed, inoculated wounds (Table 3, Fig. 4). Wounds inoculated 1 day after wounding and sprayed with 100 µg/ml of benomyl were significantly smaller ($P < 0.05$) than unsprayed, inoculated wounds (Table 3). Wounds treated at this concentration for the other five treatment times were not significantly different ($P > 0.05$) from unsprayed, inoculated wounds. There were no significant differences ($P < 0.05$) between any of the four treatments for all wounds treated 1 wk or more after wounding.

DISCUSSION

Differences in susceptibility to *N. cinnabarina* due to genetic variability in the host are well documented (7–9,13). Wehmer (13), in 1895, observed that individual trees of species usually quite susceptible to cankers caused by *N. cinnabarina* were sometimes not attacked by the fungus, even when large sources of inoculum were nearby. In addition, only a few cultivars of apple (*Malus domestica* Borkh.), Rome Beauty, Ben Davis, and Twenty Ounce, are considered susceptible to Nectria twig blight caused by *N. cinnabarina* (12). The honey locust cultivar Thornless is apparently more resistant than other cultivars to *N. cinnabarina*. The fungus was successfully recovered from all inoculated wounds on Thornless even though they were not significantly longer than the uninoculated wounds. This indicates that Thornless may be susceptible to infection by *N. cinnabarina*; however, this cultivar seems able to compartmentalize the infection quickly, and large cankers apparently do not develop.

It is evident from this experiment that infections that occur in late summer do not develop as rapidly as those occurring earlier in the season. The average canker length for the four cultivars inoculated on 28 June was 30% larger than for the late-summer inoculations, which indicates that the season of infection influences canker development. Trees inoculated on 3 September had not formed callus around the cankers before they were harvested. If the trees had not been removed, the cankers might have continued to enlarge until callus formed the next growing season. Compartmentalization of the infection appears likely after the formation of host callus (1,9).

Honey locust trees wounded in early summer remain susceptible to infection by *N. cinnabarina* for about 1 wk. Infection by *N. cinnabarina* could probably be prevented if wounds were protected during this critical period. Tree growth is greatest during the first part of the growing season. Wounds produced on honey locusts in spring and summer close more rapidly than those produced in fall or winter (10). Because *N. cinnabarina* usually causes an annual canker of honey locust (1) and successful compartmentalization is realized by formation of host callus, wounds produced late in the growing season could be expected to remain susceptible to infection longer than wounds produced in the spring.

Wound closure is not only related to season of wounding but to tree vigor (1,10). Honey locust trees under stress respond poorly to wounding and subsequent infection by *N. cinnabarina* (1). Wounds on stressed honey locusts may remain susceptible to infection by *N. cinnabarina* longer than those on vigorously growing trees. This is especially true for newly transplanted trees, which are often under severe stress. Further research is needed to elucidate the effects of stress and season of inoculation on the length of time wounds remain susceptible to infection by *N. cinnabarina*.

Concentrations of benomyl used in this study did not prevent infection of wounds on honey locust by *N. cinnabarina*, even though in vitro growth of the fungus was totally inhibited by concentrations 1/10 and 1/100 those used in the field. A recent study (12) has demonstrated that benomyl was also ineffective in reducing the incidence of twig blight of apple caused by *N. cinnabarina*. Perhaps by placing a large amount of inoculum on the wound, infection would occur regardless of the concentration of benomyl used. An inoculation technique more accurately representing natural infections may produce different results. By inoculating wounds with conidial suspensions, control may be achieved with the concentrations of benomyl used. Because wounds treated with 100 µg/ml benomyl were smaller than unsprayed wounds for 1 day after inoculation, adequate inhibition of infection may be achieved by increasing the concentrations of benomyl used.

Because *N. cinnabarina* is widely distributed and can be efficiently dispersed during wet weather (6,9), the most reliable way to control this pathogen of honey locust may be to plant resistant cultivars. The cultivar Thornless has been shown to be quite resistant to canker development and a reduction in the incidence of this canker disease might be realized by planting this cultivar. Because benomyl was ineffective in preventing infection of wounds by *N. cinnabarina* at the concentrations used in these studies, additional experimentation will be required to obtain a wound treatment that will protect trees for the short time they are susceptible after wounding.

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