

# A WOUND TREATMENT SYSTEM TO SUPPRESS CANKERS AND WOOD ROT IN TREES<sup>1</sup>

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**Abstract.** Liquid and paste formulations of Nectec, an experimental fungicidal tree wound healing product, were evaluated for the prevention or suppression of canker and wood rot in trees. A broad activity spectrum against several wood inhabiting fungi was demonstrated in the laboratory. The Nectec paste, when applied to cut ends of maple branches prior to inoculation with *Nectria cinnabarina*, significantly reduced fungus penetration. Disease control was not consistent when the cut ends of maple limbs were treated 40 days after the wounds were inoculated with *N. cinnabarina*. Application of the Nectec liquid and paste combination or the Nectec paste alone to newly wounded trunks of maple, planetree, locust, poplar and dogwood trees did not inhibit, but tended to favor callus formation. When the products were applied to cut ends of large branches of these trees, fungus penetration and staining of wood was significantly reduced after one year when compared to non-treated limb cuts. The products show promise as canker and wood rot preventatives in trees.

Invasion of tree wounds by fungi is responsible for such diseases as *Nectria* and *Botryosphaeria* canker and wood rot (2). The use of tree wound dressing products has proven to be of little benefit and often deleterious to the natural tree wound healing process (3,6). Incorporation of fungicides into wound dressing formulations has been in practice at the arborist/orchardist level in North America, but not on a commercial level (1).

Several fungicidal tree wound treatment products have been developed in Europe that reportedly prevent the invasion of pathogenic and wood rotting fungi into tree wounds (4). These products have been used to treat limb removal wounds, pedestrian and vehicular mechanical wounds to street trees, and as a treatment for decaying trunk cavities.

A Belgian company, Janssen Pharmaceutica, has developed two tree wound healing products, Nectec<sup>TM</sup> liquid and Nectec<sup>TM</sup> paste, both contain-

ing a broad spectrum fungicide and a wood preservative. The fungicide, imazalil, acts against the disease-causing organisms and the wood preservative, azaconazole, keeps out the wood decay organisms. These two fungicides have been incorporated into a paste and a liquid product.

The purpose of this research was to evaluate the performance of these wound healing products under U.S. conditions. Specifically, the objectives were to determine 1) if these products were injurious to trees either by their chemical nature or by allowing the spread of disease causing organisms during the course of applying the materials, 2) if these wound healing products could prevent disease-causing fungi from infecting fresh wounds and thereby prevent disease, and 3) if these wound-healing products could prevent or delay the invasion of wood rotting fungi into tree wounds.

## Materials and Methods

Test systems were designed to evaluate each of the objectives. Nectec paste contains 2% imazalil and 1% azaconazole and was used undiluted in these experiments. The Nectec liquid contains 100 g/l imazalil and 50 g/l azaconazole and was used diluted at 1 part Nectec liquid to 4 parts water. Also included in this study was a blank paste in which the fungicides were omitted.

To determine the activity spectrum of the Nectec paste against common wood inhabiting fungi and to see if the application of these wound-healing paints could inadvertently result in spread of the disease, the following experiment was conducted. Sawdust, collected from maple and planetrees, was steam-sterilized, moistened with half-strength potato dextrose broth, and inocu-

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lated separately with the following fungi: *Botryosphaeria*, *Ceratocystis*, *Coniothyrium*, *Cylindrosporium*, *Cytospora*, *Diplodia*, *Fusarium*, *Graphium*, *Guignardia*, *Phomopsis*, *Plenodomus*, *Pleurotus*, *Thyronectria*, and *Verticillium*. The sawdust-fungus mixtures were then mixed in 8 ml each of Nectec paste and the blank paste. The two paste mixtures were then placed on potato dextrose agar in petri dishes. A third treatment added to each plate consisted of the infested sawdust alone. Plates were then observed for fungus growth from each of the three treatments.

Two different experiments were conducted to determine if disease organisms that enter tree wounds could be controlled. To determine if the wound healing products could prevent entry of *N. cinnabarina*, a canker-causing fungus, into tree wounds, maple limbs were cut at right angles on May 18, 1990, prior to inoculation. Nectec paste and the blank paste were then applied to the appropriate cut surface. An untreated control was included and each treatment was replicated 4 times. A 2 mm potato dextrose agar disc with the actively growing fungus was placed on the treated or untreated limb surfaces. The inoculum was then covered with moist cotton and paraffin film to encourage infection. The treated limbs were examined for infection 10 and 14 wk after treatment and inoculation. Data were analyzed statistically using Duncan's multiple range test.

To determine if the Nectec liquid could eradicate fungi in an existing wound, 8 maple limbs were inoculated with *N. cinnabarina* as described above on May 18, 1990 but without the paste treatments. The parafilm covering and cotton were removed and Nectec liquid was applied 6 wk after inoculation with the fungus. The treated limbs were examined for fungus infection and sporulation 14 wk after inoculation.

To evaluate possible phytotoxic effects from the application of the tree wound healing products, cuts were made in the trunks of planetree, poplar, dogwood, locust, and maple trees on October 24, 1989. Diamond shaped pieces of bark with each side measuring approximately 2.5 cm were cut with a chisel and removed. Four treatments consisting of an untreated control, blank paste, Nectec paste, and Nectec liquid + Nectec paste were applied to

the cuts, respectively. The treatments were replicated 4 - 8 times for each tree species. Measurements of callus formation and observations for phytotoxicity were made May 18, and July 27, 1990, and July 20, 1991.

To determine if Nectec could reduce natural penetration of fungi and wood stain, limbs of planetree, poplar, dogwood, locust and maple trees were cut off 15 - 45 cm from the main trunk and three treatments, Nectec paste, Nectec liquid + Nectec paste and a non-treated control, were applied to the cut branch ends on October 10, 1989. Branch diameters ranged from 5 - 13 cm at the treatment point depending on the tree species. One year later, a 15 to 30 cm length of branch was removed from each limb treatment, subsequently sawn in half length ways, and observed for staining. Small wood discs (1.2 cm) were also removed at various distances from the treated branch end. The discs were then surface sterilized in a 0.53% sodium hypochlorite solution, incubated on potato dextrose agar and examined periodically for fungus growth. Data were analyzed statistically using Duncan's multiple range test.

## Results

The Nectec paste and sawdust study provided clear evidence of the activity of the fungicides against a wide variety of tree disease and wood rotting fungi. All fungi included in the laboratory test grew well in the sawdust when the pastes were omitted. The fungi grew in the blank paste-sawdust mixture, but at times growth was slower than in the sawdust alone treatments. When the Nectec paste was added to the sawdust, none of the fungi grew. Apparently the blank paste has little or no fungicidal activity, while the Nectec paste offers the type and spectrum of fungicidal activity needed to protect tree wounds from fungus infection.

The disease prevention study indicated that the Nectec paste could significantly delay penetration of *N. cinnabarina* into tree wounds. The wood in the blank paste and untreated wound treatments were invaded by the *N. cinnabarina* fungus which sporulated freely on the branches (Table 1). No sporulation was observed on the branches treated with the Nectec paste 10 wk after inoculation (Figure 1a). On the branches without treatment and on

those with the blank paste, the fungus sporulated extensively and had significant fungal penetration (Figure 1b, c). After 14 wk, some sporulation was observed in the Nectec paste treated limbs, however fungal entry into these branches may have occurred from the sides of the limbs, rather than from the cut ends.

Eradication of *N. cinnabarina* with Nectec liquid applied 6 wk after inoculation was not accomplished (Table 2). When disease reading were taken 14 wk after inoculation, sporulation was inhibited in only 50% of the treated branches. Infection depth was reduced in treated branches that showed sporulation.

Callus formation was evident in some diamond-shaped flush cuts in the spring, 7 months after treatment. The percentage of trees in which callus formation was initiated is given in Table 3. The amount of callus formation in the trees at this time was generally minimal. Enhancement of initial callus formation by the products was observed in all tree species but poplar.

After 2 months callus formation was again evaluated. Substantially more callus tissue had formed since the previous evaluation. Regardless of the wound treatment or tree species, the wounds exhibited approximately the same amount of closure 9 months after treatment (Table 4). No negative effects from the wound treatments were observed. In general most wounds were callused over a year later (July, 1991), however, among those that were not completely closed, the

**Table 1. Tree wound healing paint protection from *N. cinnabarina* penetration.**

Treatment	10 weeks		14 Weeks	
	Sporulation	Infection Depth (cm)	Sporulation	Infection Depth (cm)
Control	+ <sup>1</sup>	3.1 b <sup>2</sup>	+	4.1 b
Blank Paste	+	3.4 b	+	4.0 b
Nectec Paste	-	0 a	+/-	0.5a

1 Sporulation: - = no sporulation, +/- = some sporulation, and + = extensive sporulation.

2 Numbers in a column followed by the same letter are not significantly different (DMRT, p = 0.05).

**Table 2. Eradication of *N. cinnabarina* with Nectec liquid wound healing product applied 6 weeks after inoculation. \***

Treatment	Sporulation **	Infection depth (cm)
No treatment	100	5.2
Nectec Liquid	50	2.9

\* Readings were taken 14 wk after inoculation

\*\* Percentage of treated limbs with sporulation

**Table 3. Callus formation in tree wounds 7 months after treatment with wound healing products.**

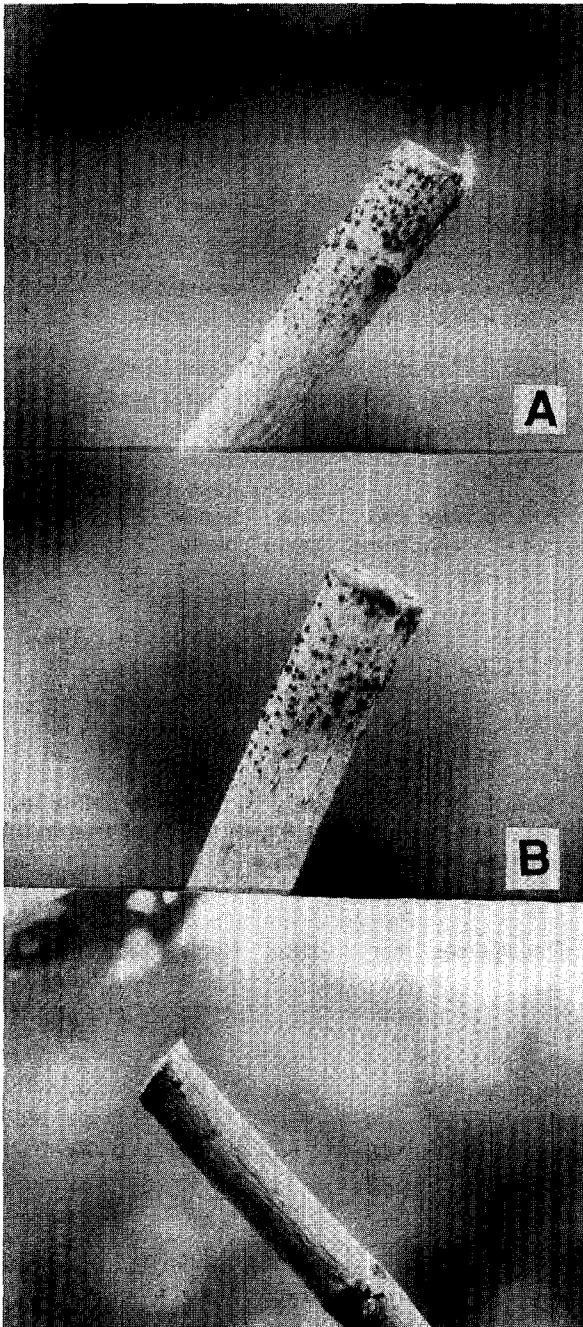
Tree species	Percent of trees with callus			
	No treatment	Blank paste	Nectec paste	Nectec liquid & paste
Dogwood	0	38	38	25
Poplar	100	100	100	100
Planetree	0	50	50	17
Maple	25	100	90	75
Locust	0	33	13	50
Mean	25	64	58	53

**Table 4. Percent of wound coverage by callus formation in tree wounds 9 months after treatment with wound healing products.**

Tree species	Percent of wound covered by callus			
	No treatment	Blank paste	Nectec paste	Nectec liquid & paste
Dogwood	2	9	5	3
Poplar	65	65	73	58
Planetree	40	40	40	40
Maple	36	30	48	46
Locust	68	66	75	70
Mean	42	41	48	43

nontreated control wounds tended to be more open (Figure 2).

The Nectec products significantly reduced staining in the cut limbs for over one year (Table 5). Untreated planetrees, locust, and poplar wounds exhibited comparable levels of discoloration. Dogwood had less stain and maple somewhat more stain. All species had reduced levels of staining from the Nectec paste alone and the



**Figure 1.** Sporulation of *N. cinnabarina* on treated and untreated maple branch wounds 10 wk after inoculation. A) Sporulation on a nontreated inoculated branch. B) Sporulation on an inoculated branch treated with paste without Nectec added. C) No sporulation on an inoculated branch treated with Nectec paste.

Nectec paste + liquid application after one year of field exposure (Figure 3).

Wood inhabiting fungi (Hyphomycetes) were isolated from Nectec treated as well as untreated wood. However, more fungi representing a wider range of species diversity were isolated from the nontreated wood samples. Clamp connection fungi (Basidiomycetes) were isolated from some nontreated wood, but not from the Nectec treated wood. The absence of the clamp connection fungi in the treated limbs may indicate a delay in the wood rotting process of Nectec treated wood.

Additional observations on unharvested treated and untreated limbs nearly two years after treatment (July, 1991) indicated that nontreated limbs generally showed much more weathering, splitting and death than the treated limbs. In a few cases Basidiomycete fruiting bodies were evident on the nontreated limbs.

### Discussion

The Nectec paste in laboratory studies had a broad spectrum of fungicidal activity against these fungi. Because of this, spread of fungi in Nectec



**Figure 2.** Wound healing in a poplar tree 1 yr after treatment. The upper untreated wound is still not completely closed while the lower two treated wounds have closed.

**Table 5. Fungal staining of cut tree limbs treated with wound healing paint after one year field exposure.**

Treatment	Plane	Wood staining (cm)			
		Maple	Dogwood	Locust	Poplar
Untreated	7.0 b <sup>1</sup>	14.3 b	3.3 b	7.0 b	7.0b
Nectec	1.3 a	0.8 a	1.9 a	1.7 a	2.5 a
Nectec L+P	0.1 a	2.3 a	0.8 a	2.0 a	2.5 a

1. Numbers in a column followed by the same letter are not significantly different (DMRT,  $p = 0.05$ ).

paste from diseased trees to healthy trees under field conditions may be inhibited since the fungi could not survive on infested sawdust carried in the paste or on application tools.

The wound healing products prevented wound infection by *N. cinnabarina* for 10 wk. This prevention was the result of the fungicide components of the products, since the wood treated with paste without fungicide was infected comparable to that of the nontreated wood. The failure to get complete disease control after 14 wk in the wound protection experiments may be misleading, since natural field inoculum levels were quite high and in-

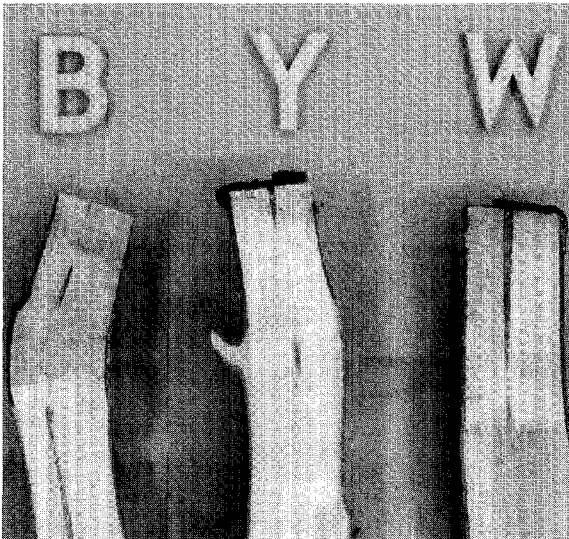
fection may have been initiated in the untreated parts of the branch.

Experiments to show the eradication properties of Nectec liquid were not conclusive. Eradication of wound-inhibiting fungi may be enhanced if the time between wounding and treatment were shortened.

Neither product tested alone or combined inhibited the natural wound healing processes. In both the limb and flush cut evaluations, wound healing was either the same as in the nontreated wood, or, in some species, enhanced by the application of the products. The sterol inhibiting nature of the antifungal compounds may provide some positive plant growth regulator effects, resulting in more rapid initiation of callus tissue. By limiting fungal activity, the fungicidal compounds may prevent inhibition of the wound healing process caused by fungal colonization of the wound surface.

Although staining in treated wood under natural conditions was significantly less than in untreated wood and fungal colonization was reduced after one year, additional studies will be needed to assess the long term advantages of the use of these products in protecting tree wounds from both pathogenic and decay producing organisms.

The Nectec paste and liquid products evaluated in this study appear to provide some advantages in the control of tree wound inhabiting fungi. The experiments demonstrate the possible use of Nectec products in reducing disease spread when wound paints are used by arborists. The eradication of canker producing fungi in new wounds and the protection of wounds against fungi until natural barriers within the tree can be



**Figure 3. Dogwood limbs 1 yr. after treatment showing staining. B) Untreated control with extensive staining. Y) Little or no staining in a branch treated with Nectec paste + Nectec liquid. W) Staining on the branch side where the Nectec paste treatment was not intact.**

established should be further studied.

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**Résumé.** Des formulations liquides et en pâte de Nectec, un produit fongicide cicatrisant expérimental pour les blessures aux arbres, étaient évalués pour la prévention ou la suppression des chancres et de la carie chez les arbres. Une large gamme d'activité sur diverses espèces de bois habitées par des champignons pathogènes était démontrée en laboratoire. Le Nectec en pâte, lorsque appliqué antérieurement à l'inoculation avec *Nectria cinnabarina* sur les surfaces de coupes de branches d'érable, réduisait significativement la pénétration par le champignon. Le contrôle de maladie n'était pas efficient lorsque les surfaces de coupes de grosses branches d'érable étaient traitées 40 jours après que les blessures furent inoculées avec *N. cinnabarina*. L'application d'une combinaison de Nectec liquide et en pâte ou de Nectec en pâte seul sur de nouvelles blessures aux troncs d'érables, de platanes, de robiniers, de peupliers et de cornouillers n'inhibait pas, mais tendait à favoriser, la formation d'un cal cicatriciel. Quand les produits étaient appliqués sur les surfaces de coupes de grosses branches de ces espèces d'arbres, la pénétration du pathogène et la coloration du bois étaient significativement réduites après un an, en comparaison avec les coupes de grosses branches non traitées. Les produits sont prometteurs en tant que prévention des chancres et de la pourriture du bois pour les arbres.

**Zusammenfassung.** Flüssige und pastöse Formulierungen von Nectec, einem im Versuchsstadium befindlichen fungizidhaltigen Wundverschlussmittel für Baumwunden, wurden orientierend untersucht hinsichtlich der Verhinderung bzw. Verminderung von Krebs und Holzfäulen an Bäumen. Ein breites Aktivitätsspektrum gegen verschiedene holzbewohnende Pilze konnte im Labor festgestellt werden. Die Nectec-Paste konnte an gekappten Ahornästen die Besiedelung durch die inokulierte *Nectria cinnabarina* Pilze signifikant reduzieren. Ein anderes Bild zeigte sich an den Kappstellen, die 40 Tage nach der Beimpfung mit *Nectria cinnabarina* behandelt wurden. Die Applikation von Nectec flüssig und pastös in Kombination oder der Paste allein auf frischen Stammwunden bei Ahorn, Platane, Robinie, Pappel und Hartriegel begünstigt die Kallusbildung, verhindert aber keine Infektion. Bei Anwendung der Produkte an großen Kappstellen war nach einem Jahr der Pilzbefall und die Verfärbung des Holzes jedoch signifikant geringer als bei vergleichbaren unbehandelten Wunden. Die Produkte lassen einen Einsatz als vorbeugendes Mittel gegen Krebs und Holzfäule erwarten.