EFFECT OF SELECTED CHEMICALS ON NON-SUBERIZED IMPERVIOUS TISSUE (NIT) FORMATION IN FRASER FIR[1]

F. H. Arthur and F. P. Hain
Department of Entomology
North Carolina State University
Raleigh, NC 27695-7613
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ABSTRACT

During the summer of 1983 and 1984, Fraser fir trees at three locations in the southern Appalachians were wounded and treated with napthalene acetic acid (NAA), pectinase and todomatuic acid. The latter two chemicals and an auxin-like compound similar to NAA are associated with balsam woolly adelgid infestations. None of the chemical treatments delayed formation of non-suberized impervious tissue (NIT) at the wound sites. In most cases, NIT formed within 21 - 27 days after wounding, even when not treated.

Key Words: Balsam woolly adelgid, *Adelges piceae,* Fraser fir, non-suberized impervious tissue, rotholz.

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INTRODUCTION

The balsam woolly adelgid (BWA) *(Adelges piceae* Ratzeburg) is a serious pest of true firs *(Abies* spp.) in North America. The BWA is native to the silver fir *(A. alba* Miller) forests of central Germany, and it causes little damage on European hosts. However, North American firs are quite susceptible to attack. The BWA was discovered in the southern Appalachians in 1956 (Amman 1962), and since that time much of the natural Fraser fir, *A. fraseri* (Pursh.) Poir, in this region have been killed (Amman 1969). When BWA infestations are concentrated on the bole of the tree, the feeding action of the adelgid stylet induces the cambium to produce abnormal xylem tissue known as "rotholz" (red wood). This wood is anatomically similar to compression wood and is relatively impervious to water flow (Balch 1962).

If the periderm tissue of coniferous bark is injured, a secondary periderm (necrophylactic periderm) is formed internal to the wound, and the necrotic cells are isolated from the healthy portion of the stem (Mullick and Jensen 1973a,b). A non-suberized impervious tissue (NIT) precedes formation of necrophylactic periderm (Mullick 1975). This is a non-specific reaction to bark injury, and Fraser fir throughout the southern Appalachians forms NIT in response to a mechanical wound (Arthur and Hain 1985).

Mullick (1975) showed that necrophylactic periderm was only partially formed at BWA feeding sites. Further studies found that NIT was not present in zones immediately adjacent to the insect stylet, and that the BWA could be producing secretory substances which inhibit or delay NIT formation (Mullick 1977). This delay may be responsible for the formation of rotholz, which can be seen as a

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continuation of the hypersensitive response to injury. This could account for the relative susceptibility of North American *Abies,* because rotholz is not present in European fir (Mitchell 1966). Most of the Fraser fir mortality has occurred in the southern portion of the range. Stands to the north of Mt. Mitchell, NC, have not been severly damaged. This differential mortality pattern may also be related to chemical interference with the NIT process.

There are only a few instances where specific compounds have been associated with BWA infestation. The saliva of the BWA apparently contains auxin-like compounds (Balch et al. 1964) and pectinase (Adams and MacAllan 1958). Puritch and Nijholt (1974) found a juvabione-like substance, todomatuic acid, in the bark of infested grand fir, *A. grandis* (Doug.) Lindl., but the origin of this substance was unclear. The effect of these chemicals on NIT formation at wound sites is unknown, and the objectives of this study were: 1) to determine if these chemicals would delay NIT formation at wound sites and 2) to determine if Fraser fir in stands experiencing either high mortality (Mt. Mitchell, NC) or little impact (Mt. Rogers, VA) due to BWA would differ in their response to chemical treatment.

MATERIALS AND METHODS

1983 Experiment

Study plots were established at Mt. Rogers, VA (elev. 1,706 m) and Mt. Mitchell, NC (elev. 1,920 m). The following technical grade chemicals were tested for their effect on NIT formation at artificial wound sites: napthalene acetic acid (NAA, Sigma Chemical Co.); pectinase (Sigma Chemical Company); and todomatuic acid (Dr. Peter Sorter, Hoffman-LaRoche, Inc.). Stock solutions of the first two chemicals were prepared by dissolving known amounts of technical material into volumetric flasks containing either distilled water (NAA) or buffered salt solution (pectinase). Serial dilutions were made by pipetting from these stock concentrations.

On 11 July, 14 mature trees (ca. 35 years old), which were not infested with BWA, were selected for testing at Mt. Rogers. A scalpel was used to make a series of shallow wounds (5 mm long, 3 mm wide, 2 mm deep) in the bark surface, in accordance with the following ordered pattern: 1 at 1.8 m from ground level, 3 at 1.5 m, 3 at 1.2 m and 1 at 0.9 m. Pure todomatuic acid (0.1 ml) was injected into the wound at 1.8 m. Solutions of 10^{-5}, 10^{-6} and 10^{-7} M NAA were injected into the wounds at 1.5 m at the rate of 0.1 ml per wound. Solutions of 20, 10 and 2 mg of pectinase per 0.1 ml were injected into the wounds at 1.2 m, and the wound at 0.9 m was the untreated control. These high concentrations were used to simulate the continual feeding action of the BWA. Only one series of wounds was made on each tree to insure that the tree would not be girdled when the samples were removed to test for NIT.

Four trees were examined for NIT 24 days after wounding, which is slightly longer than the average time required for NIT development in mid-summer (Mullick and Jensen 1976; Arthur and Hain 1985). Four trees were tested for NIT 28 days after wounding and the remaining six trees tested after 31 days. A circular bark plug with the wound in the center was removed from the tree using a #11 cork borer (dia. 12 mm). The plugs were held in a cooler and taken to the laboratory, and NIT formation at wound sites was determined by the F-F test (Mullick 1975). The bark plugs are placed cambial side down on glass rods containing 2% $FeCl_3$ for 3 days, then rinsed with water and held in 4% K $Fe(cn)_6$ for 3 days.

Eighteen trees were wounded on 28 July at Mt. Mitchell, but the treatments of 20 and 2 mg of pectinase and todomatuic acid were not replicated due to insufficient amounts of material. Therefore, the 5 wounds on each tree (1 at 1.8 m, 3 at 1.5 m and 1 at 1.2 m) received control treatments, 3 concentrations of NAA and 10 mg of pectinase, respectively. Six trees were examined for NIT 25 days after wounding, six after 28 days and the remaining six after 35 days.

1984 Experiment

Mt. Rogers and Mt. Mitchell were again used as study sites, and an additional plot was established at Roan Mt., TN (elev. 1,908 m), located midway between Mt. Mitchell and Mt. Rogers. Fir mortality at this site is more severe than mortality at Mt. Rogers, but less than that at Mt. Mitchell. A stand of European silver fir planted in Mt. Mitchell State Park was also included in the test so that the NIT response could be compared to the more susceptible Fraser fir. The new trees at Mt. Rogers and Mt. Mitchell were not used in the 1983 experiment, and the age of all the Fraser fir tested in 1984 were ca. 30 years. The silver fir had been planted in 1928.

All Fraser fir were wounded by using a hand drill to make circular wounds (dia. 8 mm, 5 mm deep) in the bark; 3 at 1.5 and 1.2 m on the same side of the tree and one control wound at 1.2 m on the opposite side. The hand drill was used to insure that all wounds would be uniform in size. Again, one series of wounds was made on each tree to minimize damage. A small piece of polyurythane sponge was placed inside the wound and secured with a pin. Solutions of 10^{-3}, 10^{-5} and 10^{-7} M NAA (per 0.2 ml) were applied to the wounds at 1.5 m by injecting the chemical into the sponges. These sponges remained moist for ca. 48 hours, and the chemicals were slowly absorbed by the tissue surrounding the wound. Solutions of 20, 10 and 2 mg of pectinase per 0.2 ml were applied in a similar manner to the wounds at 1.2 m. Both chemicals were re-applied 7 and 14 days after the trees were initially wounded (3 July at Roan Mt., 4 July at Mt. Mitchell and 8 August at Mt. Rogers).

Only 3 wounds at 1.2 m were made on the bark of the silver fir and they received the NAA treatment. The control was at the same height on the opposite side of the tree. Twelve trees were wounded at Roan Mt. and Mt. Rogers; 6 were tested for NIT after 21 days and 6 tested after 24 days. Ten Fraser fir and 10 silver fir were wounded at Mt. Mitchell; 4 were examined after 21 days and 6 after 24 days. Data for both experiments were analyzed by using Fisher's Exact Test (Steel and Torrie 1980) to compare treatments with controls.

RESULTS

When trees were tested for NIT at Mt. Rogers 24 days after wounding 1983, none of the treatments were significantly different from the control (Table 1). Although the lowest concentrations of pectinase appeared to cause some delay in NIT formation in the samples tested after 28 days, by 31 days virtually all the wounds were surrounded by NIT. Similar results were obtained at Mt. Mitchell (Table 2). The percentage of wound samples forming NIT was actually less at 28 days than at 24, and this pattern was generally consistent for all treatments, including controls.

Table 1. Percentage of samples at Mt. Rogers, VA, forming NIT at 24, 28, and 31 days after wounding on July 11, 1983.

Treatments	24 days*	28 days*	31 days†
Plain wound (control)	50	100	100
10^{-5}M NAA	50	100	100
10^{-6}M NAA	100	100	100
10^{-7}M NAA	25	50	100
20 mg of Pectinase	75	75	100
10 mg of Pectinase	75	100	100
Todomatuic acid	75	50	83

* Four samples were taken per treatment.
† Six samples were taken.

Table 2. Percentage of samples at Mt. Mitchell, NC, forming NIT 25, 28, and 35 days after wounding on July 27, 1983 (n = 6).

Treatments	25 days	28 days	35 days
Plain wound (controls)	100	67	100
10^{-5}M NAA	67	33	100
10^{-6}M NAA	83	67	100
10^{-7}M NAA	83	67	100
10 mg of Pectinase	83	83	100

The results of the 1984 experiment are summarized in Table 3. Four of the six wounds treated with 10^{-3} M NAA did not show NIT after 21 days, but the difference was not significant. There were no differences between wounds and controls tested after 24 days. Three of the four silver fir wounds treated with 10^{-7} M NAA did not show NIT, but none of the treatments had any effect on NIT formation in Fraser fir wounds at Mt. Mitchell or at Mt. Rogers.

DISCUSSION

The results of both studies indicate that the application of NAA, pectinase and todomatuic acid to artificial wounds caused little delay in NIT formation. It was expected that NAA might cause some inhibition, since BWA saliva apparently contains auxin-like compounds. The adelgid is continually injecting saliva during feeding, and high concentrations of NAA were used to try to simulate feeding effects by applying a concentrated amount of chemical to the wound tissue. The fact that NAA had little effect on NIT formation may indicate that these auxin-like compounds in BWA saliva do not inhibit the defensive response of the tree. However, continual injections of a small amount of chemical may influence NIT formation, and our negative results could have occurred because BWA attack was not mimicked accurately.

Pectinase was included in these tests because it is the only compound specifically identified as being present in the BWA, but this chemical also had little effect on NIT formation at the wound sites. It is possible that additional compounds which are unknown at the present time are responsible for delaying the NIT in susceptible fir. Our studies have shown that Fraser fir from areas

Table 3. Percentage of Fraser fir at three locations and silver fir ( ) at Mt. Mitchell forming NIT 21 and 24 days after wounding in 1984 (n = 6).

	Roan Mt.		Mt. Mitchell		Mt. Rogers	
Date trees wounded	7/3		7/4		8/1	
Treatments	21 days	24 days	21 days*	24 days	21 days	24 days
Control	83	100	75 (100)	100 (100)	83	100
10^{-3}M NAA	33	67	50 (75)	100 (100)	100	83
10^{-5}M NAA	83	83	100 (75)	83 (100)	67	83
10^{-7}M NAA	50	67	75 (25†)	100 (100)	100	100
20 mg of Pectinase	50	83	75	100	67	83
10 mg of Pectinase	83	67	50	100	100	100
2 mg of Pectinase	83	100	50	67	83	100

* Four samples per treatment.

† Significant at the 0.07 level; Fisher's Exact Test.

experiencing high mortality such as Mt. Mitchell respond in a manner similar to those firs from areas with little damage. Fraser fir in the southern Appalachians have the inherent ability to form NIT at artificial wound sites, regardless of age, location, or the injection of selected chemicals into the wounds (Arthur and Hain 1985).

Environmental factors have been shown to affect the rate of NIT formation. Warm temperatures accelerate NIT development in the summer; the process is completed within 3 weeks, while ca. 35 days is required before NIT is found at wounds made in the spring and fall (Mullick and Jensen 1976). Puritch (1973) showed that NIT development is slowed when trees are under water stress. Similar environmental influences could be operating in conjunction with chemicals secreted by the BWA during the feeding process. Future research should focus on determining which, if any, environmental factors affect susceptibility of Fraser fir by delaying NIT and necrophylactic periderm response, particularly in the areas surrounding the adelgid stylet.

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