DEVELOPMENT OF WOUND TISSUE IN THE BARK OF FRASER FIR AND ITS RELATION TO INJURY BY THE BALSAM WOOLLY ADELGID[1]

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ABSTRACT

A total of 89 Fraser fir, *Abies fraseri* Pursh. (Poir.), from seven locations in the southern Appalachians were tested for non-suberized impervious tissue (NIT) at artificial wound sites. All trees except one formed NIT in a time period that was considered normal (3 weeks), with no delays due to tree age, location or presence of balsam woolly adelgid, *Adelges piceae* (Ratz.). Although most Fraser fir react to adelgid attack by forming "rotholz" (red wood), a number of infested trees showed no visual signs of rotholz formation.

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

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INTRODUCTION

The balsam woolly adelgid (BWA), *Adelges piceae* (Ratzeburg) (Hompotera: Adelgidae) is native to the silver fir *(Abies alba* Mill) forests of central Germany. European hosts are not affected by the BWA, but it is a serious pest of *Abies* in North America. Infestations can occur either in the crown or on the bole of the tree, but stem attacks appear to be more lethal to the host. The BWA feeds on the living bark tissue, and salivary secretions injected through the stylets cause the parenchyma cells in the feeding zone to increase in number and size (Balch 1952). In addition, the cambium layer is stimulated to produce abnormal xylem tissue known as "rotholz," or redwood (Balch 1952). This wood is similar to compression wood and is relatively impervious to water and low in fiber strength. Death of the host occurs after 2 - 6 years of continued infestation (Amman 1969).

The formation of rotholz in North American fir appears to be a continuation of the defensive response to isolate the wounded bark tissue. A healthy defensive response of *Abies* to stem invasion is a dynamic process consisting of three components (Berryman 1972; Hain et al. 1983): 1) wound densing by the primary resin, 2) containment of the wound by a hypersensitive response which includes the production of secondary resins, and 3) wound healing by the formation of wound periderm.

When the periderm tissue of conifers is injured, either by biotic or abiotic means, a secondary periderm (necrophylactic periderm) is formed internal to the wound (Mullick and Jensen 1973a, b). This tissue isolates the necrotic cells of the hypersensitive response from the healthy portion of the stem and is part of the

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normal wound-healing process. Mullick (1975) identified a non-suberized impervious tissue (NIT) which precedes formation of necrophylactic periderm. This tissue is a non-specific reaction to any type of bark injury.

Fraser fir, *A. fraseri* (Pursh.) Poir., in the southern Appalachians is particularly susceptible to BWA attack. Mortality patterns are quite variable, with the greatest mortality occurring in the southern portion of the range. However, fir stands north of Mt. Mitchell, NC, have experienced considerably less mortality. Our specific objectives were: 1) to determine if inherent variation in NIT formation could account for observed mortality patterns; 2) to determine the factors which may affect rotholz formation in Fraser fir, and 3) account for the varying degrees of mortality in the northern and southern part of the range.

MATERIALS AND METHODS

During the summer of 1982, study plots were established in the following locations: 1) Mt. Mitchell, NC; 2) Mt. Richland Balsam, NC; 3) Clingman's Dome, TN; 4) Roan Mt., TN; 5) Mt. Rogers, VA; 6) Highlands, NC, and 7) Waynesville, NC. The five sites at the highest elevations (Table 1) were natural stands of Fraser fir. Site 6 was a small plantation (ca. 20 trees) of heavily infested mature Fraser fir on private land, and site 7 was a plantation of Fraser fir Christmas trees planted at the Mountain Research Station.

Individual trees were artificially wounded by using a scalpel to make several shallow cuts (5 mm long, 3 mm wide, 1 mm deep) in the bark surface. At least 10 trees were wounded at each site, but all sites were not sampled at the same time during the summer (Table 1). Trees were initially examined for NIT ca. three weeks after wounding. At this time a circular bark plug (with the wound in the center) was taken from the tree with a #11 cork borer, refrigerated in a cooler, and taken to the laboratory.

NIT formation at wound sites was determined by the F-F test as described by Mullick (1975). The top surface of the bark plug was ringed with a layer of wax, then placed cambial side down on glass rods in petri dishes containing 2% $FeCl_3$ for three days. They were then removed and held in a similar manner in dishes of 4% $KFe(CN)_6$. The glass rods provided a support for the samples so they would not become waterlogged and sink, and also insured uniform distribution of the test solutions.

The samples were removed from the second solution after 3 days, rinsed with distilled water, and dried on blotting paper. A cross section cut through the middle of the wound surface exposed a light brown zone of necrotic tissue surrounding the wound. Immediately below this tissue was a thin layer of dark brown tissue, the NIT. The remainder of the sample was colored dark blue. If NIT had not formed the entire sample was dark blue. The ages of the trees that were tested ranged from 8 to 40 years (Table 1). Both infested and unifested trees were tested for NIT.

In a second experiment, individual trees from all of the natural stands except Roan Mt. were cored through the center of the tree at DBH and the amount of rotholz was determined by visually counting the number of reddish growth rings on the core (Table 2). The cores were also x-rayed where the rotholz could be seen on the negative as a light zone of dense wood. Both of these methods were used to calculate the age at which rotholz first appeared. All of the trees that were

Table 1. NIT formation in Fraser fir stands in the southern Appalachians of varying elevation, mean tree age and degree of BWA infestation in 1982.

Location	Elevation	No. of trees tested for NIT	Age mean ± SE	Date trees wounded	Date sampled for NIT	No. of trees producing NIT
Mt. Mitchell, NC	1,920 m	5(5)*	14.8 ± 3.8	7/07	7/28	5
		10(9)	14.6 ± 3.6	7/28	8/18	10
Richland Balsam, NC	1978 m	10(10)	20.5 ± 1.1	8/12	9/04	10
Clingman's Dome, TN	1,959 m	7(2)	34.1 ± 1.0	7/15	8/04	7
		8(1)	28.0 ± 2.8	8/14	8/25	1†
Roan Mt., TN	1,907 m	10(2)	30‡	7/14	8/02	9
Mt. Rogers, VA	1,706 m	10(8)	35.1 ± 1.0	7/06	7/27	10
Highland, NC	1,219 m	4(4)	25‡	6/23	7/08	4
		4(4)	25‡	7/08	7/28	4
		5(0)	25‡	7/15	8/12	5
Waynesville, NC	853 m	10(1)	8‡	8/12	9/04	10

* ( ) Number of trees infested with a heavy population of BWA.
† These trees were sampled again on 9/8/82 (35 days); all 7 had produced NIT.
‡ These trees could not be cored, therefore tree age was estimated.

Table 2. Rotholz formation in natural stands of Fraser fir infested with BWA.

Location	No. of trees cored	Age mean ± SE	Age of first rotholz	No. of trees showing rotholz
Mt. Mitchell, NC	15	14.7 ± 1.4	10.9 ± 0.6	11(74%), n.s.†
Richland Balsam, NC	10	20.5 ± 1.2	13.4 ± 0.9	9(90%), n.s.
Clingman's Dome, TN	15*	33.6 ± 1.1	22.7 ± 2.0	7(47%), n.s.
Mt. Rogers, VA	16	34.2 ± 0.8	26.1 ± 0.6	9(56%), n.s.

* All trees supported a heavy BWA population, with the exception of those at Clingman's Dome. Only 3 trees were infested at this site.

† Percentages tested pair wise for significance using binomial confidence limits.

cored in this study were heavily infested with BWA, with the exception of those at Clingman's Dome.

RESULTS

Virtually all trees, regardless of location, age or the presence of BWA formed NIT at the artificial wound sites (Table 1). A total of 89 trees were wounded, and only one tree did not form NIT. When fir wounded in either June or July were sampled after 21 days, NIT was present. Trees were checked for NIT after a time period that was considered normal for Canadian fir, and it is possible that the trees sampled in our study produced NIT prior to the sample date. The Christmas trees at Waynesville were 8 year old at the time of treatment, and all individuals produced NIT after three weeks. The fact that NIT response was produced in the bark tissue of heavily infested trees demonstrates that the trees are capable of producing a defensive response even under the most adverse of circumstances.

Analysis of increment cores showed that a greater percentage of the young trees at Mt. Mitchell and Richland Balsam formed visually detectable rotholz in response to previous infestation, but results were not statistically signficant due to small sample size (Table 2). If rotholz is an indication of altered xylem tissue due to BWA infestation, the adelgids had been present at DBH on the trees at Mt. Mitchell and Richand Balsam for approximately 3 and 7 years, respectively. When those trees were examined during the summer of 1983 all of them were alive, though the ones at Mt. Mitchell appeared healthier. Twelve of the 15 trees from Clingman's Dome were uninfested at the time of testing, but cores from 7 of the uninfested trees showed a well-developed zone of rotholz. As mentioned above, this could be an indication of a previous infestation.

Over half (9 of 16) of the cores taken from the infested trees at Mt. Rogers did not show rotholz, however, non-visual chemical changes could have occurred (Puritch and Petty 1971). Examination of cores taken from trees that did form rotholz indicated that the stand had been infested with BWA for a least 8 years prior to 1982. No mortality due to BWA has occurred in this stand.

DISCUSSION

All *Abies* spp. are capable of producing NIT (and therefore necrophylactic periderm) under normal conditions. Fraser fir wounded in early or mid-summer require 3 - 4 weeks to complete the process of NIT development, which is similar to the results obtained for Canadian conifers (Mullick 1975; Mullick and Jensen 1976). Warm temperatures at lower elevations such as Highlands and Waynesville may accelerate the formation of NIT.

The results of our study indicate that mortality patterns in the southern Appalachians are due to factors other than inherent variability in NIT formation between different locations. Of particular importance is the fact that infested trees were still able to produce NIT at wound sites. This indicates that even severely stressed hosts are capable of producing NIT in response to a small artificial wound, regardless of location.

Environmental conditions may also affect the ability of the host to form NIT. Puritch and Mullick (1975) showed that rates of NIT formation were slowed when trees were under water stress. Since BWA attack effectively places the tree in a

state of physiological drought (Puritch 1971), NIT formation could be delayed. In our study there appeared to be no differences in the development of NIT in infested trees *vs.* that of uninfested trees. Several of the infested trees at Highlands later died, but even they formed NIT at the same rate as healthy trees.

The results show that most Fraser fir react to artificial wounding by forming NIT, and appear to produce rotholz in response to BWA attack. As previously mentioned this abnormal xylem tissue is low in permeability, and the reduced water flow is one of the factors contributing to host mortality. It has been suggested that under conditions of heavy infestation NIT formation may be delayed or even absent, and mortality may result from BWA activity interfering with the defensive reactions of the host by producing secretory substances which delay NIT formation, or by stimulating the host to produce chemicals which prevent the normal defensive reactions (Mullick 1977).

This delay could account for the susceptibility of North America *Abies,* because European firs do not form rotholz in response to BWA attack (Mitchell 1966). Therefore, rotholz formation can be viewed as a continuation of the hypersensitive response (Mullick 1977). Although the average adult BWA stylet is only 1 - 2 mm long (Balch 1952) the effects of feeding actions may reach deeper into the bark, thus simulating a deeper wound. The autonomous process by which sapwood conduction is blocked and the necrotic tissue isolated from the remainder of the xylem appears to describe rotholz formation. Under conditions of heavy infestation this reaction can be occurring at literally thousands of points on the stem. Eventually the entire xylem could be rendered non-conductive. Therefore, the defensive mechanisms are functioning somewhat normally, but the tree is essentially killing itself by producing non-conductive rotholz in place of normal xylem cells.

There is less mortality of Fraser fir in the northern portion of the range; trees in the vicinity of Mt. Rogers have apparently been infested for at least 20 years, yet there is little damage (Lambert et al. 1980). Differences in host physiology could account for this mortality pattern. Future research should focus on determining if in fact there are differences between tolerant and susceptible trees in NIT formation at the point of insect attack, as well as determing if differences in rotholz formation are also important in conferring BWA tolerance.

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