ADELGID HOST INTERACTIONS WITH SPECIAL REFERENCE TO THE BALSAM WOOLLY ADELGID IN NORTH AMERICA

F.P. HAIN[1] and R.G. HOLLINGSWORTH,[1]
F.H. ARTHUR,[2]
F. SANCHEZ,[3]
and R.K. ROSS[4]

[1]Department of Entomology
North Carolina State University
Raleigh, NC 27695 U.S.A.

[2]USDA Stored Products Insects R & D Lab
Savannah, GA 31403 U.S.A.

[3]Department of Chemistry
North Carolina State University
Raleigh, NC 27695 U.S.A.

[4]Department of Genetics
North Carolina State University
Raleigh, NC 27695 U.S.A.

INTRODUCTION

The objectives of this paper are: 1) to provide a general overview of adelgid biology and the various host relationships; 2) to review the current knowledge of the interactions of the balsam woolly adelgid, *Adelges piceae* (an introduced pest), and its North American hosts; 3) to report on the most recent research involving the interactions of this adelgid with Fraser fir, *Abies fraseri*, in the southern Appalachians; and 4) to provide a synthesis of the above in the form of an hypothesis regarding host tolerance for the balsam woolly adelgid.

Adelgids are conifer-feeding insects in the super-family Aphidoidea of the order Homoptera. They are frequently referred to as aphids and have similar features such as a soft body and membranous wings. Both feed on plant sap. However adelgids are placed in a separate family, Adelgidae, because they differ from true aphids in having short antennal segments, a reduced wing venation, glandular body surface, no siphunculi, and because all the female forms are oviparous (Carter 1971).

The life cycles of species within the Adelgidae is either holocyclic, requiring a primary and secondary host, or anholocyclic, occurring only on one host. In the holocyclic life cycle the primary host is always a spruce (*Picea*) and the secondary host is a conifer of another genus such as fir (*Abies*), larch (*Larix*), pine (*Pinus*), Douglas-fir (*Pseudotsuga*), or hemlock (*Tsuga*).

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The holocyclic life cycle (e.g. *Adelges laricis*), as described by Carter (1971), is shown in Fig. 1. On the primary host, eggs are laid by a fertilized female (apterous sexuales). The egg hatches into a fundatrix nymph that settles on the current year's shoot. After overwintering, feeding activity is closely synchronized with the flow of sap in the spring. The mature fundatrix females lay a large cluster of parthenogenetic eggs which hatch into gallicolae nymphs. As the nymphs feed on the sap of the young tissue the characteristic adelgid gall forms. During the summer the deformed needle bases which form most of the gall become separated, and the winged gallicola is released. Throughout the summer the gallicolae migrate to the secondary host (*Larix* in the case of *A. laricis*) where they settle on the needles and lay eggs.

The parthenogenetic eggs of the gallicolae give rise to sistens and the first generation on the secondary host begins (Fig. 1). After overwintering as first or second instar sistens, the sistens mature in the spring and lay large clusters of eggs which hatch into progrediens nymphs. Two morphs of the progrediens nymph develop simultaneously into apterous oviparae and alate sexuparae. The latter migrate back to the new needles of spruce and lay eggs which hatch as males and females of the sexuales generation, and the entire life cycle is completed. The apterous progrediens may produce additional generations of progrediens or sistens during the summer. They overwinter as sistens and give rise to sexuales and progrediens the following spring.

Anholocyclic adelgids do not have all the morphs in Fig. 1 and reproduction is by parthenogenesis exclusively. The life cycle may be confined to any one of the conifer genera. Carter (1971) described four types of anholocyclic adelgids. Two types are on spruce: one has gall-forming morphs that have the appearance of the fundatrices and gallicolae (e.g. *Adelges abietis*), the other type has only apterous forms and no galls are formed (e.g. *Pineus pineoides*). The other two types occur on the secondary host and produce sistens and progrediens; one type may produce sexuparae that are incapable of producing males (e.g. *P. pini*), the other type does not produce sexuparae although alates are produced which give rise to sistens (e.g. *A. piceae*).

The focus of this paper, *Adelges piceae*, the balsam woolly adelgid, infests *Abies* species and is believed to be native to the silver fir, *A. alba*, forests of central Europe. The adelgid was introduced into North America around 1900. European firs are not seriously affected by this insect, but North American firs frequently experience either crown dieback or tree death or both. Adelgid-caused damage has been extensive and, at times, intense.

Europeans place the true fir-infesting adelgids in the genus *Dreyfusia* (Borner 1908). The British and North Americans follow a classification that places all the sub-family Adelginae into two genera, *Pineus* and *Adelges*, based on the number of abdominal spiracles (Annand 1928, Carter 1971). This latter classification is used here. Three subspecies of *A. piceae* have been identified on the basis of morphometric analysis (Foottit and Mackauer 1980, 1983): *A. piceae piceae*, Pacific Northwest, British Columbia, and southeastern United States; *A. piceae canadensis*, eastern Canada and New England; and *A. piceae occidentalis*, British Columbia.

The balsam woolly adelgid was introduced into Maine and Nova Scotia in 1908 on nursery stock and became established on balsam fir, *A. balsamea* (Balch 1952). The adelgid was discovered in the southern Appalachians in 1955[1] infesting Fraser fir, *A. fraseri*, on Mt. Mitchell, North Carolina. Since then it has been found on Fraser fir in Virginia and Tennessee, and on bracted balsam fir, *A. balsamea* var. *phanerolepis*, in Virginia and West Virginia (Amman 1962). Balsam fir has the most extensive

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[1]J.S. Boyce. Memorandum of October 7, 1955, to North Carolina National Forests, Toecane Ranger District, Burnsville, North Carolina. Southeastern Forest Experiment Station, Asheville, North Carolina. As reported in Amman 1966.

Generalized Adelgid Life Cycle

Stage	Season
Fertilized Eggs	Fall
↓ Fundatrix nymphs	Winter
↓ Fundatrix mature females	Spring
↓ Parthenogenetic Eggs	
↓ Gallicolae nymphs	Summer
↓ Emerge as alates (fly to secondary host)	Fall
↓ Parthogenetic Eggs	Fall
↓ Sistens nymphs (neosistentes)	Winter
↓ Sistens mature females	Spring
↓ Alate sexuparae (fly back to primary host) → ↓ Sexuales; ↓ Apterous oviparae (remain on secondary host)	Summer

Figure 1. Holocyclic and anholocyclic life cycles of adelgids.

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range of all the North American true firs, occurring from Newfoundland to Alberta, Labrador to Pennsylvania; however the adelgid is only found on balsam fir in New England (as far south as New York), Newfoundland, Nova Scotia, New Brunswick, and Quebec (Greenbank 1970, Mitchell et al. 1970). A separate introduction occurred in the Pacific Northwest, where the principal hosts are grand fir (*A. grandis*), subalpine fir (*A. lasiocarpa*), and Pacific silver fir (*A. amabilis*) (Mitchell 1966).

The life cycle of the balsam woolly adelgid consists of the egg, three larval instars, and the adult. The first instar, or crawler stage, is about 0.4 mm in length, and is the only form capable of movement (Fig. 2). Within 24 to 48 hours after hatching, the crawler locates a suitable feeding site, inserts its stylets into the bark of the host, and transforms, without molting, into a flattened, wax-fringed resting stage (neosistentes). The insect is sessile, remaining permanently attached to the feeding site which is usually a bark lenticel or other roughened area of the main stem, branch, twig node, or bud base (Bryant 1974). Dispersal occurs when the crawlers or eggs are passively transported by wind, birds, or other animals. The second and third instars are about 0.50 and 0.65 mm in length, respectively. They closely resemble the adult and are covered with secretions of waxy threads that appear as a dense white wool mass. The second instar through the adult are considered the sistentes. The adult females are about 0.80 mm in length, dark purple to black, nearly spherical, and wingless. As many as 248 amber-colored eggs are laid within the woolly mass. The dense woolly secretions provide protection for all of the life stages except the crawler (Balch 1952). The progrediens stage has

been observed in Europe and the Maritime Provinces of Canada (Mitchell et al. 1970), although it is rare. There are winged and wingless forms of the progredientes. The wingless form is similar to the sistentes, while the winged form has conspicuous membranous wings and five-segmented antennae and generally lack wax pores. The progrediens stage gives rise to the neosistens stage.

The winter is passed as a dormant first instar nymph, and this generation is referred to as hiemosistens. Development is completed in the spring (Balch 1952, Greenbank 1970). The first instar nymphs of the second generation (aestivosisten) also undergo a dormant period (summer aestivation) ranging from 2 to 8 weeks (Amman 1969). At lower altitudes or warmer climates, additional generations may be produced (Arthur and Hain 1984, Mitchell et al. 1961). A third generation usually results from faster development, and a partial fourth may occur during years of extremely early development.

The amount of damage sustained by an infested tree is related to the size of the attacking adelgid population, to the part(s) being attacked, and to the physiological state of the tree. Adelgids generally concentrate in the outer portions of tree crowns or on the main stem and large branches. The precise location of the infestation depends on the tree species and geographic location. Stem infestations usually cause more damage and tree mortality than do crown infestations. Heavy stem attacks give the lower bole a white-washed appearance because of the conspicuous presence of white woolly masses. In some cases, stem infestations may virtually disappear after a few years without killing the tree--this is especially true in Europe. However, many North American firs are killed within 2 to 6 years of a sustained infestation.

The anatomical, structural, and physiological changes in host tissue caused by an *A. piceae* infestation include the production of abnormal wood (rotholz) in the xylem tissues, an increase in outer bark thickness, bark resinosis and sometimes copious resin flow from within the feeding zone, and the production of juvabione and juvabione-like compounds. The stimulation of the cambium to produce rotholz is particularly intriguing, since the physical presence of the stylets only extend to the living bark cells. In young and thin bark tissue, the stylets occasionally reach the phloem but do not enter it (Balch 1952).

Rotholz is usually reddish in color and resembles compression wood (Balch 1952, Busby 1964, Varty 1956). The intensity of the red coloration varies from barely noticeable to dark red. The xylem tissue from infested fir contains higher amounts of ray tissue (Mitchell 1967, Smith 1967), thickened cell walls, shorter tracheids (Doerksen and Mitchell 1965, Foulger 1968), a reduced number of conducting pits in the tracheids (Puritch and Petty 1971), and encrusted pit membranes that resemble those of normal heartwood (Puritch and Johnson 1971).

Water flow through sapwood samples from infested trees was greatly reduced (Mitchell 1967), almost to the level of non-conduction (Puritch 1971). There are no distinguishable differences between normal heartwood and the heartwood formed as a response to adelgid attack (Puritch 1971, 1977, Puritch and Petty 1971). Since the cambium and ray cells are not physically damaged, it appears that the adelgid merely enhances the normal pattern of heartwood formation. An infestation is also associated with the production of chemical compounds in the sapwood (Puritch 1977). In contrast to uninfested trees, water transport in infested trees is limited to narrower bands of early wood and does not ascend as high (Mitchell 1967). The tree is in a state of physiological drought. As fluid movement is impaired, photosynthesis and respiration are reduced, contributing to the death of the host. European firs do not form rotholz in response to attack (Mitchell 1966), which may explain the innocuous status of the pest.

When the periderm tissue of conifers is injured a layer of impervious tissue (Biggs 1985, Mullick 1975) followed by necrophylactic (secondary) periderm is formed internal to the wound (Mullick and Jensen 1973a, 1973b). This impervious tissue isolates the necrotic cells of the wound response from the unwounded portion of the stem. In a susceptible response to an adelgid infestation, the formation

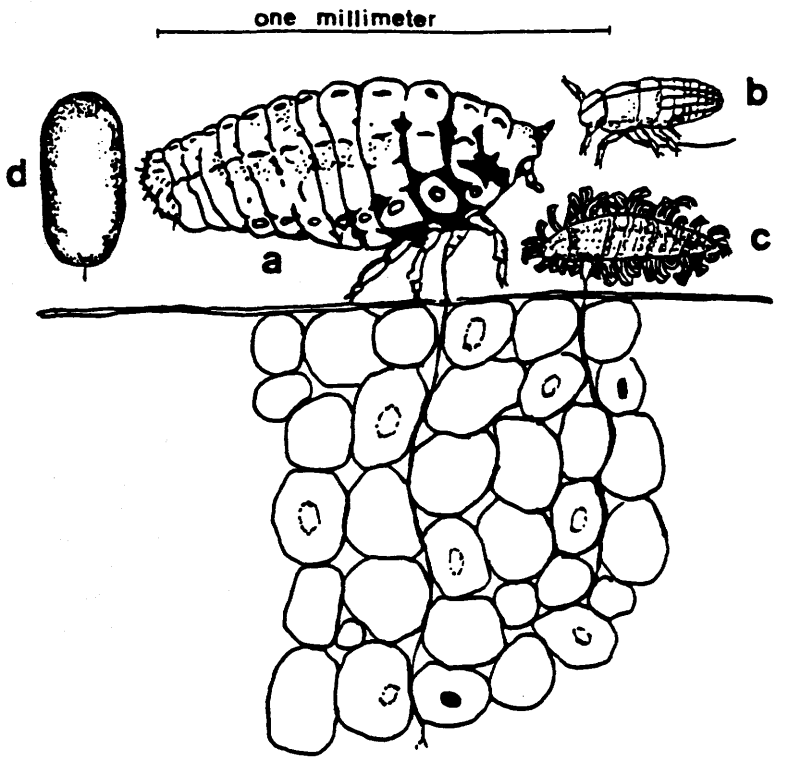

Figure 2. Life stages of the balsam woolly adelgid: a) adult with stylets piercing bark tissue, b) crawler, c) neosisten with wax fringe and stylets piercing bark tissue, and d) egg.

of the impervious layer may be inhibited (Mullick 1975, 1977), perhaps as a result of stylet secretions. The wound response continues into deeper tissue layers and ultimately into the xylem, where rotholz is formed. By contrast, tolerant firs may complete the formation of the impervious tissue without delay and thus avoid any permanent damage to the underlying cells. Eventually nutrients in the outer bark become exhausted and the adelgid disappears leaving the tree relatively unharmed.

The wound response of some fir, especially grand fir, *Abies grandis*, produces such copious amounts of resin that it flows down the bole. Grand firs are considered one of the more resistant North American fir species to adelgid attack with only 20 to 30 percent of the infested trees dying (Mitchell 1966). Adelgid-infested grand firs do not always produce rotholz (Puritch and Petty 1971), although the anatomical and chemical features of the xylem are altered and permeability reduced (Puritch 1973, 1977, Puritch and Petty 1971, Puritch and Johnson 1971).

Also juvabione-like substances may be produced by the host in response to an adelgid infestation. The substances were not found in uninfested fir, nor were they found in infested fir except in the vicinity of the adelgid (Puritch and Nijholt 1974). Perhaps the juvabione-like compounds interfere with the metabolism of the insect and render the tree resistant to a prolonged infestation.

We have reviewed the general life history of adelgids that attack spruce and fir, focusing on an adelgid introduced into North America, the balsam woolly adelgid. Research into the interactions of the adelgid with its new hosts has shown that the infested trees respond, in varying degrees, by the production of rotholz in the xylem, wound periderm in the bark, resins, and juvabione-like substances.

The objective of our research at North Carolina State University has been to clarify the importance of each of the reaction components (rotholz, wound periderm, resin, and juvabione-like substances) to the tolerance or susceptibility of Fraser fir to the balsam woolly adelgid. This paper will review that work and propose a hypothesis for the cause of tree susceptibility or tolerance.

METHODS

Most of these studies were conducted on Fraser fir, *Abies fraseri*, in the southern Appalachians of North Carolina. However, some comparative studies were also conducted on European silver fir, *A. alba*, in the Black Forest of Germany.

Rotholz Formation and Water Potential

Several methods were used to determine the presence of rotholz. The first was wood coloration. Wood cores were taken from opposite sides of trees. When a growth ring from both cores showed a high proportion of latewood, giving it a reddish coloration, the ring was considered to contain rotholz. Some cores were also treated with perchloric acid (a heartwood indicator) to enhance the difference in coloration. Other cores were examined by electron microscopy. Rotholz is anatomically similar to compression wood (Balch 1952), having thick-walled tracheids which are circular (rather than rectangular) in cross-section (Doerksen and Mitchell 1965, Timell 1986), and encrusted pit membranes which appear similar to those of heartwood (Puritch and Johnson 1971).

Water potential of individual trees was measured using a Scholander pressure chamber (Scholander et al. 1965) and all readings were taken at dawn. When necessary, extension clippers were used to remove a branch from the upper crown and a 2-year-old twig was cut from the branch for use in the chamber.

Sapwood flow rates were measured through Fraser fir stems previously infested by the balsam woolly adelgid and containing rotholz. Four pairs of Fraser fir were cut and removed from an infested Christmas tree plantation. Each pair included one tree which had been heavily attacked by the adelgid and a second tree which did not show symptoms of a heavy infestation. Ten-cm long bole sections were placed with the bottom portion in a reservoir of distilled water, and a partial vacuum pressure of 10 psi was applied to the upper portion. The amount of water drawn through the sample during two consecutive 2-minute intervals was recorded. Water dyed with food color was then drawn through the sample at 27 psi. Flow rates were calculated on a per unit area of sapwood basis. Sapwood was defined to include all wood except heartwood.

Wound Periderm and Bark Thickness

The formation of wound periderm was evaluated by artificially wounding individual trees using a scalpel to make several shallow cuts (5 mm x 3 mm x 1 mm deep) in the bark surface. Trees were examined for suberized impervious tissue by removing a circular bark plug (with the wound in the center) about 3 weeks after wounding. The formation of impervious tissue was determined by the F-F test (Mullick 1975). The bark plugs are held phloem side down in a 2 percent solution of $FeCl_3$ for 3 days, and then in a 4 percent solution of $KFe(CN)_6$ for an additional 3 days. After drying, a radial cut through the bark plug exposes a light brown zone of necrotic tissue surrounding the wound. Immediately below this tissue is a thin layer of dark brown tissue, the suberized impervious tissue. The remainder of the plug will be colored dark blue. If the impervious layer is not formed, the entire sample will be dark blue.

Outer bark thickness was determined also using the F-F test. Unwounded plugs were taken from two sides of a tree. After staining, the samples were cut, and the maximum depth of any impermeable layer present (i.e. outer bark) was measured.

Monoterpene Analysis

We quantified the volatile fraction of resin, the monoterpenes, that was present within the necrotic tissue surrounding an artificial wound produced as described above. The monoterpenes found in healthy Fraser fir have been identified (Zavarin and Snajberk 1965, 1972, Thor and Bennett 1974), but there was no information concerning the monoterpene fraction of wounded tissue.

Wounded trees were sampled using a cork borer (dia. 11 mm) to remove a circular bark plug from the wound surface. All samples were placed in individual vials containing 4 ml of n-pentane, placed in a container of dry ice and taken to the laboratory and held at 0°C until analyzed. Control samples of unwounded tissue were handled in the same manner to determine the constitutive monoterpene composition.

The monoterpene content of each sample was analyzed by gas chromatography using one microliter of paracymene as an internal standard. All monoterpenes were identified by comparing their retention times with that of known standards. The amount of each monoterpene was quantified using a recording integrator. After this analysis, the dry weight of each bark sample was determined.

Juvabione Analysis

Juvabione analyses have been reported earlier (Puritch and Nijholt 1974, Manville 1975, 1976, Manville and Bock 1977, Manville and Kriz 1977). However, our procedures were different. We analyzed air-dried 5/8 inch woodcores rather than cross-sections of felled trees. The most recent annual ring and any annual rings showing evidence of rotholz were analyzed. The wood sections were ground up and subjected to a Soxhlet extraction (24 hr) with methanol. The extract was eluted through a C18 Waters sep-pak with 10 ml of methanol, and then analyzed by capillary gas chromatography. The retention times were compared to those of pure samples of extracts of Douglas-fir supplied by Dr. John Manville (verified by GC-MS).

Genetic Variations of Fraser Fir

Because of the wide variation in mortality caused by the balsam wooly adelgid within the natural range of Fraser fir, a related study assessed the genetic architecture of the southern Appalachian endemic Fraser fir by analyzing seven polymorphic allozyme loci found in five major mountain populations. Mature mother trees and their respective progeny were sampled in groups of 5 to 10 at the peaks of the mountains, and at other representative locations within populations. Samples consisted of cones with ripe seed.

Cleaned seed were germinated on moist filter paper and then subjected to electrophoretic analysis. Megagametophytes and embryos were excised from the seed coat, crushed separately, and inserted into the vertical slice of a gel. Of the approximately 25 enzymes tested in preliminary trials, 12 were chosen for scoring because of their good resolution in embryos and gametophytic, or their polymorphic character.

RESULTS AND DISCUSSION

Rotholz Formation and Water Potential

Although rotholz production is a common occurrence in infested fir species of North America, not all infested trees show the visual signs of rotholz. However, the lack of any visible indications of rotholz does not necessarily indicate that the xylem has been unaltered by the infestation. Heavily infested grand firs, grown as exotics in Scotland, were found to contain no visible evidence of rotholz, yet the sapwood of these trees was only one-twentieth as permeable as sapwood sampled from nearby

uninfested trees. The adelgid-altered wood stained the same color as heartwood (Puritch 1971). The reduced permeability was due to a combination of tracheid pit membrane encrustation and accelerated embolism (Puritch and Johnson 1971, Puritch and Petty 1971).

Rotholz formation in infested Fraser fir was evaluated by studying wood cores taken from six sites near Mt. Rogers, Virginia, and five sites in the Black Mountains of North Carolina. Average adelgid densities were higher for trees sampled at Mt. Rogers. At moderate-to-high infestation densities, the proportion of trees producing rotholz was similar between locations. However, a significantly higher proportion of trees from the Black Mountains produced abnormal wood when lightly infested.

Pressure chamber measurements, made on cut shoots of Fraser fir, add additional evidence that adelgid infestations might interfere with water transport through the sapwood. Measurements taken in June showed that infested trees at Mt. Mitchell and Roan Mountain, North Carolina, had lower xylem pressure potentials (an indication of poorer water status) than uninfested controls, while the infested and uninfested trees at Mt. Rogers, Virginia, did not differ from one another. In mid-summer, there were no significant differences at any site. However, infested trees at all locations showed lower water potentials than uninfested trees when measured in September (Arthur and Hain 1986). Increment cores taken from infested trees at all three sites contained visible rotholz, and nearly all cores from uninfested trees at Mt. Mitchell also contained rotholz. This suggests that the uninfested trees at Mt. Mitchell may have recovered from an earlier infestation. As September was a low-rainfall month in the southern Appalachians during this study, these results may indicate that the physiological effects of adelgid-caused damage are more severe during periods of low rainfall.

In a comparative study in the Black Forest of Germany, xylem and phloem potential measurements were made on ca. 10-year-old silver fir infested with a closely related adelgid, *Adelges* (= *Dreyfusia*) *nordmanniana*, which infests branches and twigs. Trees were classified as uninfested, lightly infested, and heavily infested. Table 1 shows that both the phloem and xylem potential readings of the infested trees were considerably higher than that of the uninfested. In fact, the phloem of many of the infested trees had liquid oozing out of just one or two spots rather than the entire phloem area. When tested for rotholz with perchloric acid, many of the smaller twigs tested positive. *A. nordmanniana* was introduced into the Black Forest and is considered a pest. It is also capable of causing rotholz formation on the thin-barked twigs, while the native *A. piceae* does not cause rotholz formation on the thicker-barked boles of silver fir.

When sapwood flow rates were measured through Fraser fir stems previously infested by the adelgid, areas of rotholz generally did not transport dye. Rotholz inclusion is associated with reduced sapwood permeability and an increase in the proportional area of heartwood (Hollingsworth and Hain,

Table 1. Water potential readings of *Abies alba* infested with *Dreyfusia nordmanniana*

Degree of infestation	Water potential (-bars)	
	Xylem	Phloem
Heavily infested (n = 16)	10.09 ± 3.27	1.46 ± 1.06
Lightly infested (n = 10)	7.95 ± 1.96	1.75 ± 2.55
Uninfested (n = 15)	8.03 ± 2.67	0.73 ± 0.44

unpubl. data). These results lend support to the hypothesis that rotholz leads to water stress in the crowns of infested trees.

Wound Periderm and Bark Thickness

Adelgid attack causes chemical and structural changes within the tissue of susceptible hosts (Balch 1952). Initially the number and size of bark parenchyma cells increase within the area surrounding the insect's stylets. Within a year, these enlarged cells become surrounded by purplish cork cells which are produced by secondary phellogen. The parenchyma cells disintegrate, and the area becomes infiltrated with resin and completely enclosed within cork tissue. In heavily infested stems, this secondary cork cambium forms two or more millimeters beneath the surface of the bark, forming a localized, thickened layer of dead outer bark cells which physically prevents feeding by future generations.

The response described above is typical of conifers injured by biotic or abiotic factors. It serves to isolate the damaged area from the living tissues of the bark, while adding additional layers of protection in the damaged area. Many woody plants (Mullick 1977) exhibit this type of non-specific response when living bark cells are damaged.

In fir trees, the wound response initially involves the formation of a layer of suberized impervious tissue (Mullick 1975) to wall-off the damaged tissue, followed by the formation of a reddish-purple sequent (necrophylactic) periderm internal to the impervious layer. To determine if inherent variation in the formation of the layer of suberized impervious tissue could account for the observed mortality patterns of Fraser fir in the southern Appalachians, we tested 89 firs from seven locations for the complete formation of the suberized impervious tissue at artificial wound sites. The F-F test (Mullick 1975) showed that all the trees except one formed the impervious layer in a time period that was considered normal (3 weeks) during the growing season, with no delays due to tree age, location, or presence of balsam woolly adelgids.

There are a few instances where specific chemicals have been associated with a balsam woolly adelgid attack. The saliva of the adelgid contains auxin-like compounds (Balch et al. 1964) and pectinase (Adams and McAllan 1958). A juvabione-like substance, todomatuic acid, was found in the bark of infested grand fir (Puritch and Nijholt 1974). To determine the effect of these chemicals on the formation of the suberized impervious tissue at wound sites, we artificially wounded 86 fir trees (including 10 European silver fir planted at Mt. Mitchell) at three locations in the southern Appalachians. The wounds were treated with either napthalene acetic acid (NAA), pectinase, or todomatuic acid (Arthur and Hain 1985). None of the chemicals delayed formation of the impervious tissue.

Observations on other fir species indicate that outer bark, formed in response to attack by the balsam woolly adelgid, is frequently associated with recovery of infested trees (Brower 1947, Pschorn-Walcher and Zwölfer 1958, Schooley and Bryant 1978). Apparently the outer bark forms a physical barrier to adelgid feeding, or forces the insect to feed on other portions of the tree. Of 94 trees measured for bark thickness at Mt. Rogers, and 99 at Mitchell, a higher proportion of infested trees had outer bark thickness greater than 1.5 mm (the average length of a balsam woolly adelgid stylet) in comparison to uninfested trees. This was especially true of trees with a high infestation density and for trees with old wool only. Trees with old wool were recently infested (the woolly masses had not washed off yet), but not currently infested.

There was some indication that the "outer bark" detected in the Mt. Mitchell trees (defined by its impermeability to staining solutions) was not completely impermeable to the passage of moisture from the inner bark. When a new periderm layer is formed internal to the old one, all of the bark tissue between the two layers should gradually desiccate, becoming hard and compressed. Because of its distinct texture, this tissue is easily identified, and was classed as "dry outer bark" at the time we

measured the thickness of the impermeable tissue. From Mt. Rogers, there were 23 trees which contained a measurable amount of outer bark, and 16 of these also contained "dry outer bark" as a proportion of the total outer bark thickness. By contrast, only 8 of 37 Mt. Mitchell trees with outer bark also contained "dry outer bark."

Average thickness of the dry outer bark was considerably greater for the silver fir trees from Germany, regardless of infestation class, than for Fraser fir trees of the southern Appalachians (Hollingsworth and Hain, unpubl. data). Silver fir trees generally contained more moist outer bark as well. It seems probable that silver fir trees naturally produce more outer bark than Fraser fir. An alternative explanation is that many of the silver fir studied may have formed the outer bark in response to previous infestations.

Monoterpene Deposition

The bark of 22 mature Fraser fir from Mt. Mitchell and Mt. Rogers was artificially wounded. Two to six weeks after wounding, wound tissue and unwounded tissue were sampled and analyzed for monoterpenes.

The results of the bark wound reaction study showed that there were no differences in total monoterpene content between wound tissue and normal bark tissue at either Mt. Mitchell or Mt. Rogers. However, there were differences between the two sites. Trees from Mt. Rogers had lower amounts of delta-3-carene relative to Mt. Mitchell. The variation between trees at Mt. Mitchell (which probably reflects historical harvesting and planting practices) could indicate differences in susceptibility to adelgid attack. Because Mt. Rogers trees have experienced little adelgid-caused mortality, it has been hypothesized (Arthur and Hain 1987) that those Mt. Mitchell trees that more closely resemble Mt. Rogers trees (and may in fact be a Mt. Rogers seed source) are more tolerant of an adelgid infestation.

In a similar study in Germany's Black Forest, monoterpene composition of wounded and unwounded tissue was compared between infested trees from a low elevation site and healthy and declining trees at a high elevation site. The cause of the decline at the high elevation site was unclear (perhaps it was influenced by atmospheric deposition) but none of the trees showed any sign of a balsam woolly adelgid infestation.

As seen in Fig. 3, the amount of monoterpenes found in the phloem tissue was considerably higher in the trees from the infested site. This was especially true for the wounded tissue. The monoterpene composition (Fig. 4) for the wounded tissue of the infested trees was similar to that of the wounded tissue of the declining trees at the high elevation. The amount (Fig. 5) of limonene in the unwounded tissue of the infested trees was also very high compared to the other trees.

It appears that the silver fir of the Black Forest, as opposed to Fraser fir, do respond to wounding of the phloem tissue by an accumulation of monoterpenes around the wound site.

It is not clear whether monoterpenes contribute to the tolerance mechanisms of fir trees infested by the balsam woolly adelgid. However, for other conifer species, monoterpene contents are thought to be important for host resistance to a number of insect pests and diseases (Rockwood 1973, Shrimpton 1973).

Juvabione

In addition to monoterpenes, some members of the genus *Abies* produce sesquiterpenoids which act as insect juvenile hormones (Slama and Williams 1965). Two such compounds, todomatuic and dehydrotodomatuic acids, have been identified from Pacific silver fir and grand fir infested with the adelgid (Puritch and Nijholt 1974). These compounds were found only in the vicinity of attack and

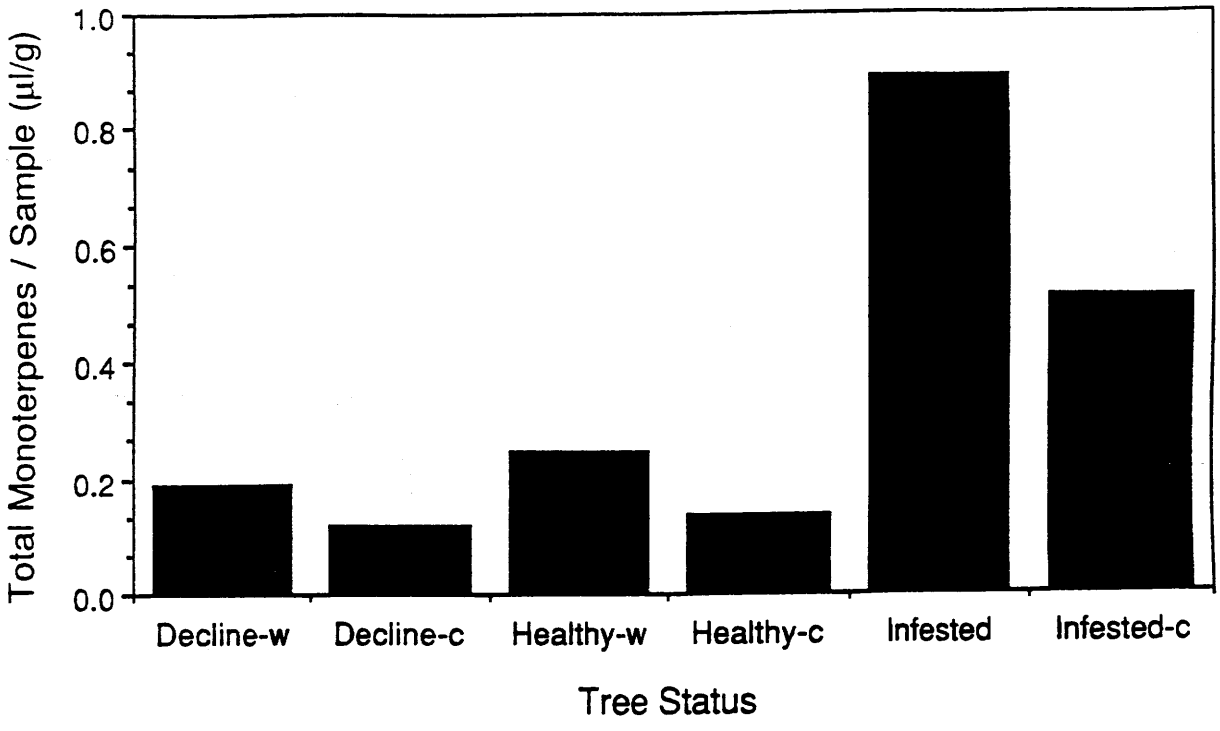

Figure 3. Total monoterpenes of European silver fir in the Black Forest of Germany. Decline-W = wound tissue from an uninfested declining tree, Decline-C = unwounded control tissue, Healthy-W = wound tissue from an uninfested healthy tree, Healthy-C = unwounded control tissue, Infested = wound tissue from an infested tree, and Infested-C = unwounded control tissue.

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were not present in uninfested trees. Todomatuic acid and juvabione have been isolated from seedlings of infested Fraser fir (Sanchez, unpubl. data).

The trees at the two sites in the Black Forest were also examined for the presence of juvabione and todomatuic acid (Table 2). Both compounds were detected but in very small amounts, especially the todomatuic acid. The concentration of these compounds was about 100-fold less than that detected in the infested Fraser fir seedlings (Sanchez, unpubl. data), and about 10-fold less than that detected in some of the infested mature grand and Pacific silver fir (Puritch and Nijholt 1974). However, the compounds were detected in the mature European silver fir, even in uninfested trees.

It has been hypothesized that juvabione-related compounds may interfere with adelgid development (Puritch and Nijholt 1974). The effective level of concentration, however, is unknown.

Genetic Variation of Fraser Fir

This study (Ross 1988) assessed the genetic architecture of Fraser fir throughout its restricted geographic range by analyzing seven polymorphic allozyme loci. The five major mountain populations differed slightly but significantly from each other with respect to allele frequency for both mature tree and progeny populations. The Mt. Rogers trees had the most extreme allele frequencies and appear to be an outlier among the five mountain populations. These differences may be due to local, restricted

Table 2. Presence of juvabione and todomatuic acid in *Abies alba*

Type	Age ± SE	Bark thickness MM ± SE	ul/g ± SE Juvabione	Todomatuic acid
Declining	96 ± 8	8.25 ± 1.66	1.60 ± 0.38	0.13 ± 0.20
Healthy	91 ± 5	9.39 ± 1.68	1.34 ± 0.13	0.03 ± 0.02
Infested	55 ± 7	6.28 ± 0.96	1.88 ± 0.52	0.01 ± 0.01

mating and genetic drift. Therefore, the distinctiveness of the Mt. Rogers fir trees is demonstrated genetically as well as entomologically with regards to adelgid tolerance. However, it has not been demonstrated that the genetic distinctiveness is in any way related to adelgid tolerance.

SUMMARY AND HYPOTHESIS

The balsam woolly adelgid's life cycle is anholocyclic with parthenogenetic reproduction on fir trees only. The adelgid is native to the fir trees of central Europe but has been introduced into North America. European firs are not seriously affected, but North American firs frequently experience either crown dieback or tree death, or both. Fraser fir, in the southern Appalachians, is one of the most susceptible North American species to an adelgid infestation. Research into the interactions of the adelgid with its new North American hosts has shown that infested trees respond by the production of rotholz in the xylem tissue, wound periderm in the bark tissue, resins, and juvabione substances. The objective of this paper has been to clarify the importance of each of the reaction components to the tolerance or susceptibility of Fraser fir to the adelgid. Comparisons with European silver fir were also made, and the genetic architecture of the major mountain populations of Fraser fir was examined.

Balsam woolly adelgid infestations interfere with water transport through the sapwood and undoubtedly interfere with phloem transport as well. Whether rotholz is visible or not, water potential measurements were lower in infested trees. In trees with rotholz, dye transport within the rotholz zone did not occur and there was an increase in the proportional area of heartwood. A significantly higher proportion of trees from the Black Mountains produced rotholz when lightly infested than did trees from Mt. Rogers, suggesting a greater sensitivity to an adelgid infestation in some mountain populations of Fraser fir.

Outer bark formed in response to attack by the balsam woolly adelgid appears to be associated with recovery of infested trees. Infested trees, at both Mt. Mitchell and Mt. Rogers, had a higher proportion of trees with an outer bark thickness greater than 1.5 mm. However, a higher proportion of Mt. Rogers trees contained "dry outer bark" as a proportion of the total outer bark thickness. The average thickness of the dry and moist outer bark of the silver fir trees in Germany was considerably greater than that of Fraser fir, regardless of infestation class. This suggests that the formation of outer bark, especially dry outer bark, is an important tolerance mechanism for mature trees infested by the adelgid.

While differences in monoterpene composition of bark tissue did exist between Mt. Mitchell and Mt. Rogers trees, there was no significant accumulation of monoterpenes in wounded bark tissue. This is in contrast to the silver fir of the Black Forest where wound tissue surrounding an artificial wound had a significantly higher accumulation of monoterpenes than unwounded tissue, especially in currently infested trees.

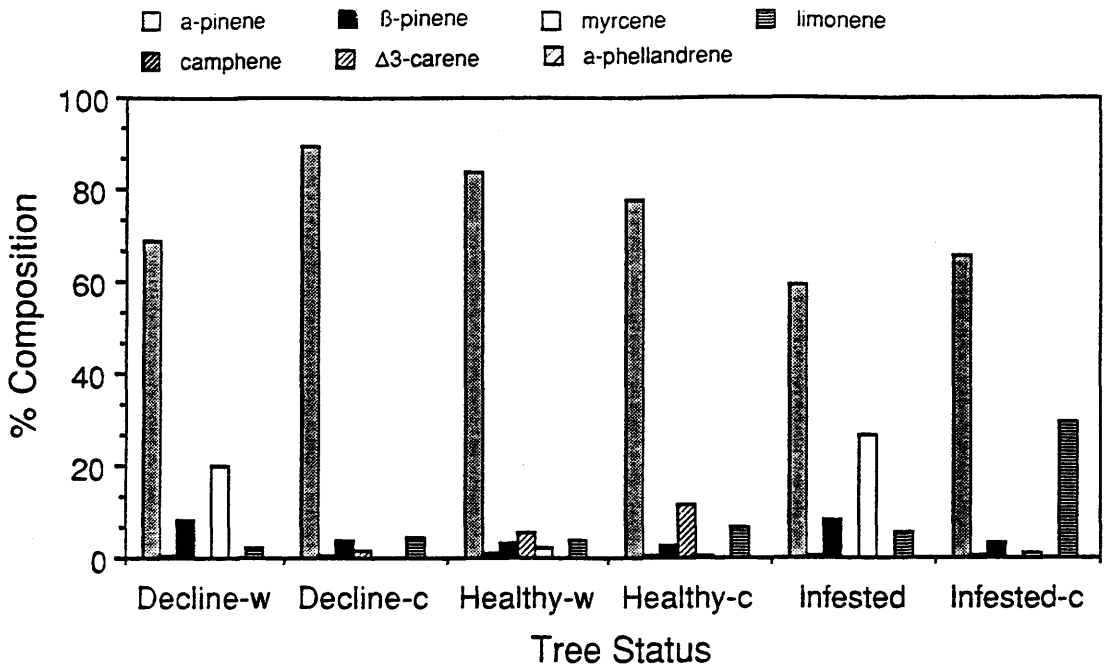

Figure 4. Composition of monoterpenes of European silver fir in the Black Forest of Germany. See Fig. 3 for description.

Juvabione-related compounds have been found in several fir species and may cause abnormal adelgid development. In some North American fir species the compounds were only found in infested trees. Infested Fraser fir seedlings had extremely high quantities of these compounds. Both infested and uninfested mature silver fir of Germany had detectable amounts of the chemicals, but in very low quantities. If these compounds do interfere with adelgid development, the effective concentration level is not known.

The balsam woolly adelgid studies in the southern Appalachians have demonstrated that the fir population at Mt. Rogers is unique with regards to adelgid/fir interactions. A genetic study of allele frequencies has also demonstrated the distinctiveness of the Mt. Rogers fir population. The Mt. Rogers trees had the most extreme allele frequencies and appear to be an outlier among the fir populations of the southern Appalachians.

Although our knowledge of the balsam woolly adelgid/Fraser fir interaction is incomplete, we know enough about the system to offer the following hypotheses about susceptibility, resistance, and tolerance of fir to an adelgid infestation.

In a susceptible fir there is an inadequate response tissue to the infestation within the bark. There is no immediate accumulation of monoterpenes or juvabione-related compounds, and an inadequate accumulation of outer bark. Both the phloem and the current xylem rings become non-translocating because of chemical and structural changes within the tissue. Continuous infestation over a period of years results in accumulation of non-translocating tissue that eventually kills the tree, especially during periods of drought.

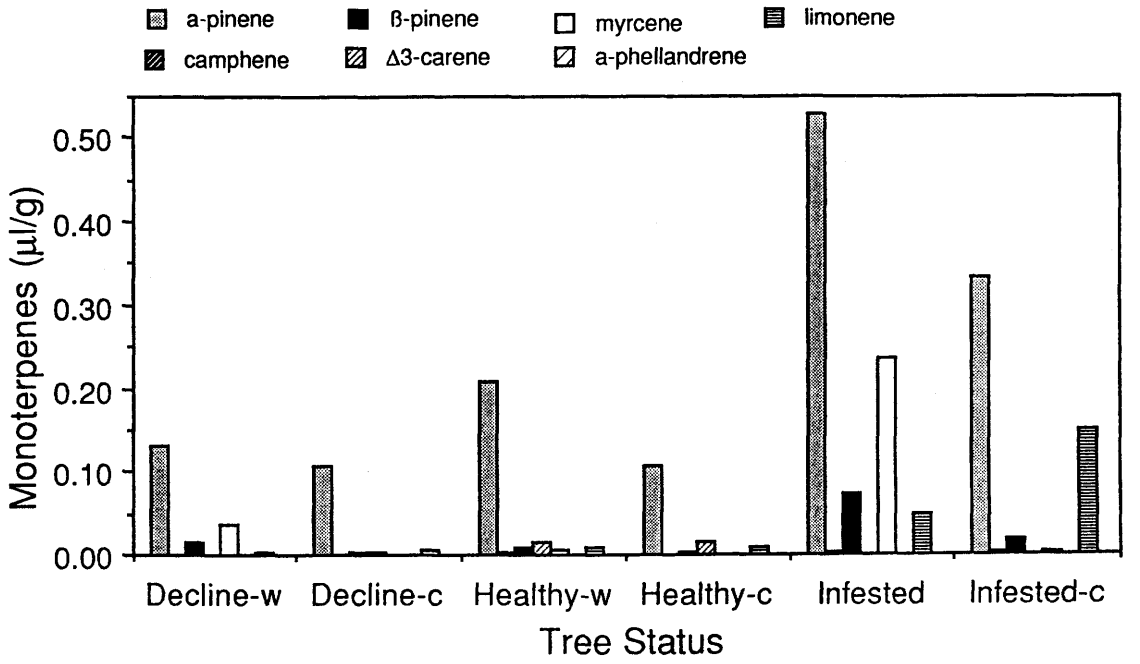

Figure 5. Amounts of monoterpenes of European silver fir in the Black Forest of Germany. See Fig. 3 for description.

In a resistant or tolerant fir there is a rapid accumulation of monoterpene and juvabione-related compounds surrounding the invasion site. In mature trees the chemical accumulation serves to inhibit survival and development of the adelgid. In younger trees, especially seedlings, the accumulation of juvabione-related compounds is so high that abnormal adelgid development prevents the insect from successfully reproducing. Older trees, which accumulate less juvabione-related compounds, begin to produce secondary periderm and a thick layer of outer bark. As the outer bark thickens and dries, it becomes unsuitable as an adelgid substrate. The insect either abandons the host, or abandons that part of the host, to infest another tree or another part of the same tree. If the process occurs rapidly, minimum damage is inflicted and the tree survives.

Future work will examine the above hypotheses in greater detail.

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