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Investigations of Balsam Woolly Aphid - Fraser Fir Interaction: Feeding Site Characteristics and Wound Response

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To the Graduate Council:

I am submitting herewith a dissertation written by Christopher Eagar entitled "Investigations of Balsam Woolly Aphid - Fraser Fir Interaction: Feeding Site Characteristics and Wound Response." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Ronald L. Hay, Major Professor

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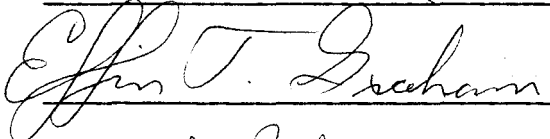
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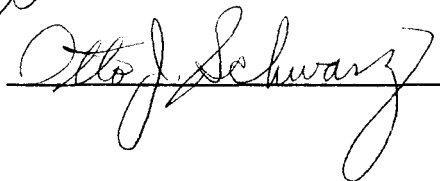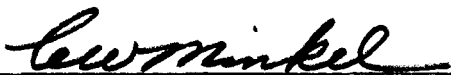
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INVESTIGATIONS OF BALSAM WOOLLY APHID-FRASER FIR

INTERACTION: FEEDING SITE CHARACTERISTICS

AND WOUND RESPONSE

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Christopher Eagar

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ABSTRACT

Young, vigorously growing Fraser firs have exhibited a lower degree of susceptibility to balsam woolly aphid infestations than mature, mixed stands. This study investigated the relationship between balsam woolly aphid feeding site preference and bark morphological features, plus how these morphological features varied with stem size, age, growth rate, percent live crown and stand structure characteristics. Investigations were also conducted on the wound healing processes within the bark of Fraser fir as well as on how the aphid might interfere with these processes. Sampling was performed in stands considered to be representative of anticipated conditions for the next generation of Fraser fir following the death of existing mature Fraser fir.

For successful feeding the balsam woolly aphid required modification of the tight, smooth, gray bark characteristic of young, vigorously growing Fraser fir trees. These modifications were in the from of lenticels and splitting of the bark. Fir trees with slow growth rates associated with high stand densities had rougher bark and more lenticels per unit area than trees growing in open, less competitive conditions.

The wound healing processes within the bark were studied by observing the rate of formation on non-suberized impervious tissue and necrophylactic periderm following mechanical wounding. Sampling was conducted along an elevational gradient and a stand density gradient. Open-grown trees at low elevations showed the fastest rates of healing

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(17 days), whereas trees growing at the highest elevations required 26 days to heal. At the same elevation, open-grown trees formed necrophylactic periderm an average of 5 days sooner than forest-grown trees. Wounding combined with injection of selected plant growth substances (some of which are suspected of being secreted by the aphid while feeding) was also investigated. All treatments utilizing auxin-like compounds (indole-3-acetic acid and naphthaleneacetic acid) required 11 more days for periderm formation than for the control of mechanical wounding only. Treatments utilizing a gibberellin and a cytokinin formed necrophylactic periderm at the same rate as the control. Additionally, histological examination of aphid feeding sites failed to reveal a single case of necrophylactic periderm formation around the feeding zone. Thus, the balsam woolly aphid was able to inhibit the normal defense mechanism of Fraser firs following penetration of the living bark tissue by the insect's stylet.

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CHAPTER 1

INTRODUCTION

Infestations of the balsam woolly aphid (Adelges piceae Ratz.) in the Southern Appalachian spruce-fir forests have concerned scientists, resource managers, and recreationists. This pest is a tiny, sucking insect that infests the bark of true firs (Abies spp.) causing mortality much out of proportion to its size. Fraser fir (Abies fraseri (Pursh) Poir.) is quickly killed by the balsam woolly aphid, with the time from initial infestation to death of the host between three to nine years, depending on the size and vigor of the tree (Amman and Speers 1965). The intensity of the host reaction combined with the phenomenal reproductive potential of the aphid has already created significant changes in the species composition and structure of the Southern Appalachian spruce-fir forests.

The balsam woolly aphid is native to Europe and was first identified in North America in 1908 on balsam fir (Abies balsamea (L.) Mill.) in Maine (Kotinsky 1916). The insect probably arrived in North America prior to 1900 on imported nursery stock with separate introductions in southern Nova Scotia and Maine (Balch 1952). Having spread throughout the Maritime Provinces and New England the aphid has caused damage and mortality to balsam fir over thousands of hectares (Schooley and Bryant 1978).

A separate introduction on the west coast of North America resulted in the aphid being identified on noble fir (Abies procera

Rehd.) and grand fir (Abies grandis (Dougl.) Lindl.) near San Francisco in 1928 (Annand 1928). Firs in the Willamette Valley were infested by 1930, and by the 1950's the aphid was established throughout the Pacific Northwest from the coast to the crest of the Cascade Mountains (Mitchell 1966).

The balsam woolly aphid was first detected in the Southern Appalachians on Mount Mitchell, North Carolina, in 1957 (Speers 1958). Subsequent surveys revealed that there were 11,000 dead firs and the aphid had spread throughout the entire 3,035 hectares of Fraser fir type on Mount Mitchell (Nagel 1959). High mortality and widespread aphid distribution indicated aphid establishment prior to 1957, perhaps as early as 1940. The Mount Mitchell infestation then spread to the Fraser fir communities throughout the Southern Appalachians.

Balsam woolly aphids could not have chosen to invade a better peak in order to further their population expansion. Mount Mitchell, the tallest peak in the East, is centrally located to all Fraser fir in the Southern Appalachians. The Black Mountains, of which Mount Mitchell is a part, have a north-south orientation in an otherwise southwest-northeast oriented chain. Therefore, Mount Mitchell is a distinctively tall peak in a continuum of tall peaks providing relatively easy dissemination to the other Southern Appalachian Fraser fir stands.

Balsam woolly aphids were detected in 1962 on Roan Mountain (Ciesla and Buchanan 1962), which is located 32 kilometer N 15° E of Mount Mitchell. Grandfather Mountain was found to have aphids in

1963; it is 48 kilometers N 50° E from Mount Mitchell. The same year, aphids were found on Mount Sterling in Great Smoky Mountains National Park. Mount Sterling is 64.4 kilometer and S 85° W of Mount Mitchell (Ciesla et al. 1963). In subsequent years balsam woolly aphids were found in all Fraser fir stands.

Initial infestations on prominent mountain masses in the Great Smoky Mountains were located at the lower elevational limits of Fraser fir (Eagar 1978). On mountains with a longer history of balsam woolly aphid activity, dead trees occurred at low to middle elevations (1300 to 1700 meters). This was followed by a zone of heavy infestations with variable mortality and then by a gradual decrease in infestation intensity with increasing elevation to the summit. Johnson (1977) found that the largest Fraser fir in a stand supported the highest aphid populations. From their tall crowns, these trees provided optimum loci to spread infestations upslope.

During earlier studies of the infestation characteristics of the balsam woolly aphid in the Great Smoky Mountains, it was found that young, dense, pure, even-aged stands of Fraser fir had low aphid populations (Eagar 1978). These stands suffered little aphid-caused damage even though Fraser fir growing in surrounding mixed, old-growth spruce-fir stands had succumbed several years earlier. Additional field observations identified two other groups of Fraser fir which exhibited reduced susceptibility to aphid attack: small (less than one meter tall) understory trees, and trees growing in the open without competition. These trees had one common characteristic which

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might have been related to reduced aphid colonization--their bark surface differed from mature Fraser fir growing within stands. The current study was conducted to better understand host/aphid interactions with specific reference to factors associated with aphid feeding site selection.

During the course of this project, a workshop on the balsam woolly aphid in the Southern Appalachians was sponsored by the Southern Appalachian Research/Resource Management Cooperative. Discussions with researchers from Canada resulted in an expansion of the scope of the study to include aspects of inherent defense mechanisms within the bark of Fraser fir, plus the role that chemicals suspected of being secreted into the bark by the feeding aphid had in altering host defense mechanisms. Therefore, these study objectives were developed:

1. Determine bark morphological features associated with balsam woolly aphid feeding site selection on trees growing under a range of stand conditions and a range of tree ages within each stand.

2. Evaluate a) the response within the bark of Fraser fir to wounding, b) the wound response around balsam woolly aphid feeding sites, and c) the effect of plant growth substances on the rate of wound healing.

CHAPTER 2

LITERATURE REVIEW

I. BIOLOGY AND ECOLOGY OF THE BALSAM WOOLLY APHID

Adelges piceae (Ratz.) is in the order Homoptera, the superfamily
Aphidoidea, family Phylloxeridae, and sub-family Adelginae. There are
11 species of adelgids that infest Abies, all of which are holarctic
in origin. Two of the 11 species, A. piceae and A. nusslini (Borner),
have inadvertently been introduced into North America. A. nusslini,
indigenous to Europe, has a limited distribution in North America
where it has not seriously damaged its hosts. In Europe this species
has caused limited economic damage, primarily to European silver fir
(Abies alba (L) Miller) (Bryant 1974). A. piceae is relatively
innocuous in Europe but it is extremely damaging in North America. It
is believed that A. piceae evolved from A. nusslini following the
movement of the latter species from the Caucasian region to Europe.
In Europe the two species apparently occupy different ecological
niches with A. piceae feeding on the trunk and A. nusslini feeding on
the twigs and needles (Balch 1952). This pattern has not been
observed in North America.

Life Cycle and Development of Adelges piceae

The fir adelgids exhibit complex polymorphic life cycles which
often include alternation of hosts, with spruce (Picea spp.) being the
primary host and the true firs (Abies spp.) being the secondary host.

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Sexual reproduction is associated with the primary host and parthenogenesis with the secondary host. Throughout its range in North America the balsam woolly aphid has evolved the capacity to produce successive generations on the secondary host and is not found in the sexual or migrant form (Balch 1952).

The life cycle of the balsam woolly aphid consists of an egg, three larval instars, and the adult. The following description of each stage is based on examinations made in eastern Canada by Balch (1952).

Eggs are oval, light purplish-brown, about 0.4 millimeters long, and are attached behind the stationary parent by a silken thread. As the egg matures the color changes to orange-brown. Emergence occurs at the opposite end of attachment with the larvae emerging head first.

The first instar (neosisten) is the only form capable of movement. This "crawler" is about 0.4 millimeters long with an oval, ventrally flattened body. Upon emerging from the egg, its color is light purplish-brown. Once a suitable feeding site is located, the stylet is inserted into the bark and feeding begins. This developing instar becomes purplish-black and small tuffs of wax threads appear on its sides. The insect remains at this location for the rest of its life.

The second instar has a broader body and is about 0.5 millimeters long. The color remains the same as the first instar; however, the entire body becomes covered with long, curling, white wax threads which give the balsam woolly aphid its characteristic woolly

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appearance. During this stage the legs and antennae atrophy.

The third instar is about 0.65 millimeters long with all other characteristics similar to the second instar.

Adults have a hemispherical body that is slightly longer than it is wide. The color is still purplish-black and the aphid is about 0.8 millimeters long. By this time the wax threads have reached their maximum development and provide easy recognition.

Seasonal Development

Balsam woolly aphid populations in North America are capable of producing a minimum of two generations per year. The overwintering generation is called the hiemosistens, and the generation that completes its life cycle during the summer is known as the aestivosistens. There is little biological or morphological difference between the two generations.

Hiemosistens. All life-stages from eggs to adults have been observed in late autumn; however, only the first instar larvae that have inserted their stylets and become dormant survive the winter. Insect activity begins when the host tree starts its annual growth cycle, but not all aphids begin feeding at the same time. Balch (1952) reported 20 days variation between individuals in the time that aphids broke dormancy on the same tree. Vigorous trees are the first to initiate growth in the spring, therefore, these are the first to show signs of aphid feeding. This is indicated by swelling of the body and the formation of a small, clear drop of honey dew at the

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anus. In the southern Appalachians, Amman (1962) found that dormant first instars began feeding as early as April 10 and as late as May 15 during the 1960 growing season.

After feeding begins in the spring, the first adults occur during late April to early May. Reproduction begins when the adult is 2 or 3 days old and continues for at least 5 weeks. Eggs require about 9 to 12 days of incubation, but that varies according to temperature and humidity (Balch 1952; Amman 1968; Greenbank 1970).

Aestivosistens. Within a few hours of hatching the first instar of the aestivosistens usually finds a suitable feeding site close to the parent. Two or 3 days after the stylet is inserted into the bark, the aphid enters diapause for a duration of 3 to 8 weeks. Fluctuating temperatures are required to break diapause and finish metamorphosis (Atkins 1972). The adult aestivosistens are less fecund than the adult hiemosistens and produce only about half as many offspring.

Seasonal history. In eastern Canada the balsam woolly aphid has two generations per year (Balch 1952). However, Bryant (1971) reported that a third generation was completed in crown infestations in Newfoundland, Canada. This third generation was comprised of the offspring of progredientes, a usually rare, non-diapause form which develops from the first eggs of the hiemosistentes. Progredientes are not an important factor in the population dynamics of the aphid in other regions of North America.

The greatest intraregional variation in seasonal development is encountered in the Pacific Northwest due to the wide range of

elevation and environmental conditions available there. Four

generations were reported for infestations located in the lowlands,

three generations were found in the intermediate elevations, and only

two generations occurred at the highest elevations (Mitchell, et al.

1961)

In the southern Appalachians the balsam woolly aphid may have as

many as three generations per year but two generations are more common

(Amman 1962).

Factors affecting development. Temperature and humidity are the

principle factors that affect the developmental rate of the balsam

woolly aphid. Nymphal development can occur between 7° C and 25° C,

with a regime of fluctuating temperature in the middle segment of this

range being most beneficial for development (Atkins 1972; Greenbank

1970). Amman (1970) found that cool temperature and low humidity had

an adverse affect on hatching and subsequent nymphal development.

When exposed to these conditions eggs suffered desiccation. Low

temperatures reduce crawler vigor, affecting their ability to locate

suitable feeding sites (Atkins and Hall 1969). Greenbank (1970) found

that the duration of oviposition varied inversely with temperature,

and low temperature produced the highest fecundities. Other factors

such as the physiological condition of the host also affected the

reproduction potential of individual aphids.

Reproductive potential. Balsam woolly aphid reproductive rates

are influenced by the weather conditions throughout the entire life

cycle of the insect, the vigor of the host tree, the amount of

protection provided at the feeding site, and the individual aphid.
Balch (1952) reported that the hiemosistens averaged about 100 eggs
per adult with a maximum of 248 and that the aestivosistens averaged
about 50 eggs per adult with a maximum of 105 eggs. Survival rates
varied with environmental and host factors, but averaged about 60
percent for both generations.

Behavior of the Aphid

Dispersal. Being wingless and except for the first instar
incapable of active travel, the balsam woolly aphid is dependent on
passive movement. The principal dispersal vector is air currents;
crawlers, and occasionally eggs, are readily transported by wind.
Balch (1952) found that they were transported more than 90 meters by
surface winds and several kilometers by vertical air currents in
eastern Canada. In the mountainous Southern Appalachians, wind has
been responsible for the movement of the aphid of distances up to 68
kilometers (Amman 1966).

Atkins and Hall (1969) found that crawlers located on trees
around the perimeter of a stand were responsible for the majority of
the long distance dispersal of the aphid. Within a stand that has a
relatively closed canopy the movement of aphid crawlers is from crown-
to-crown or from crown-to-stem, but rarely from stem-to-stem
(Greenbank 1970).

Gravity is an important means by which infestations spread within
a stand. It is also the primary manner by which fir seedlings and
saplings become infested.

Humans were responsible for the initial introduction of the insect into North America. It is possible that they have also contributed to the further spread of the aphid by movement of nursery stock and Christmas trees. Some may have unknowingly transported crawlers or eggs on clothes or vehicles.

Woods and Atkins (1967) determined that small animals were occasionally responsible for the movement of aphids, but phorsey was not a common method of dispersal.

Activity. Upon hatching aphid crawlers experience a period of wandering about the host. Such movement was not influenced by light or gravity, but it appeared only a random search for a suitable feeding site (Atkins and Hall 1969).

Crawlers are capable of moving distances of 100 feet, and they can remain active for as long as 8 days. However, they usually settle close to their sedentary parent within 2 or 3 hours of hatching. Temperature influences the length of crawler activity with high temperatures shortening the time that crawlers actively move. Crawlers tend to drop from trees at a higher rate during periods of high temperatures (Atkins and Hall 1969).

Atkins (1972) reported that in laboratory tests groups of crawlers tended to settle more quickly than isolated individuals. Therefore, the spreading process of the aphid may be facilitated by the synchronous dispersal and arrival of aphids. The occurrence of numerous crawlers at the same time was dependent on environmental conditions during the incubation period.

Feeding. The balsam woolly aphid feeds intercellularly. The stylet, which is about four times as long as the body, penetrates through the phellem and into the phelloderm where the insect feeds in parenchyma. The stylet is partially withdrawn and re-inserted during feeding; its direction is apparently determined by aphid senses and not solely by the path of least resistance (Balch 1952).

The first instar nymph enters diapause following the insertion of the stylet. Prior to diapause a salivary substance secreted from the tip of the maxillae forms a sheath around the stylet. This salivary substance is also secreted during feeding; one probable purpose is the modification of the feeding site in a manner that facilitates the uptake of needed nutrients (Balch et al. 1964). Pectinase activity has been reported for balsam woolly aphid saliva (Adams and McAllan 1958). Additional information on the effects of balsam woolly aphid feeding will be reviewed in the section on host/pest interactions.

Ecology and Population Dynamics of the Aphid

Environmental factors. High ambient air temperatures are not fatal to the balsam woolly aphid (Balch 1952). In fact, consistently warmer than normal temperatures within a region would benefit the population, because the increased developmental rate induced by the warmer temperature could increase the number of generations per year which would greatly add to the growth of the population. However, high bark surface temperatures, caused by direct sunlight on a darkened object, are fatal to the adult and all nymphal stages causing

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death by desiccation. The second and third instars as well as the adult are afforded some protection from solar radiation by the wool that covers their bodies. Death of aphids by exposure to direct sunlight is insignificant in terms of overall population dynamics.

Rain can dislodge and wash away eggs. It also mats down the wool and occasionally completely washes it away, increasing the probability of death from desiccation. Aphids are protected from drowning when immersed in water by their wax secretions (Atkins and Woods 1968).

Extremely cold temperatures limit population growth in the more northerly latitudes but they are not a factor in the Southern Appalachian. All aphid stages except the overwintering first instar are killed by prolonged exposure to temperatures below 0° C and instantly by temperatures below -20° C (Balch 1952; Greenbank 1970). Overwintering first instars require a period of gradual exposure to colder temperatures in order to develop cold-hardiness before they can withstand extremely low temperatures of -25° C to -34° C (Greenbank 1970). The minimum lethal temperature for the overwintering first instar is $-34.4\ ^o$ C (Balch 1952). Amman (1967) reported that an overnight temperature of -34° C on Mount Mitchell, North Carolina caused higher than normal mortality, but there was no indication that significant control of aphid populations resulted from these rare, low temperatures in the Southern Appalachians. Under normal weather conditions all aphid stages have adaptations which enhance their survival.

Biotic factors. The balsam woolly aphid in North America is free
of damaging parasites and diseases. Introduction in eastern Canada,
the Pacific Northwest, and the Southern Appalachians of foreign and
native insect predators of the aphid had little effect on population
dynamics (Balch 1952; Brown and Clark 1960; Amman 1961; Mitchell 1962;
Mitchell and Wright 1967; Amman 1970; Amman and Speers 1971).
Ineffective synchronization of predator-prey life cycles, poor
searching ability of the predators, the high reproductive capacity of
the balsam woolly aphid, and the rapid death of the host tree were all
factors in the failure to reduce populations.

The carrying capacity of the host tree is the only biotic factor
that significantly affected the upper limits of aphid populations in
the Southern Appalachians. This carrying capacity was surpassed in a
comparatively short time, resulting in a sharp reduction in the aphid
population followed by death of the host (Amman 1970).

Applied factors. Lindane (gamma isomer of benzene hexachloride)
has been the primary chemical for control of the balsam woolly aphid;
however, it is of little practical use in forest conditions since the
entire bole of the tree must be sprayed to the point of saturation.
Lindane has been used to protect critical, high-use recreational areas
on Mount Mitchell during the 1960's (Ciesla et al. 1963) and on Roan
Mountain beginning in 1971 (Ward et al. 1973; Johnson et al. 1980).
At both locations, spraying involved application of 1/8 percent
lindane within a 200 foot wide zone on both sides of the primary
access roads. The use of lindane has come under increased scrutiny by

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the Environmental Protection Agency due to its persistence within the environment. Nash and Woolson (1967) reported a 10 percent average residue in the soil 14 years after application of 56 and 224 kg/ha to several soil types at Beltsville, Maryland. Lindane is broken down within the soil by microorganisms (MacRae et al. 1967). An evaluation on Mount Mitchell showed high initial concentrations in the litter layer, followed by slow movement into the soil with a peak concentration at 1.5 years after application and a gradual decrease over the next 3.5 years (Jackson et al. 1974). Tests for lindane in animals and stream water were negative.

Other chemicals which have been shown effective in killing larval and adult balsam woolly aphids under field conditions are propoxur (Baygon), permethrin (Ambush and Pounce), chlorpyrifos (Dursban) and chlorpyrifos-methyl (Reldan) (Hopewell and Bryant 1969; Carrow et al. 1977; Hastings et al. 1979; Puritch et al. 1980). As with lindane, these insecticides must be applied from within the stand to each individual stem. Most also have limited ovicidal effect, thereby necessitating multiple applications per year. Several of these compounds are toxic to birds and mammals and their use in natural areas is restricted.

An alternative to the use of highly toxic and/or persistent petro-chemicals is the potassium salt of oleic acid (Puritch 1975). Fatty acids are natural constituents of plants and animals, biodegradable and when used properly, they are low in phytotoxicity. They have biocidal effect on certain soft bodied insects, including

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the balsam woolly aphid. The exact mechanism of death is not certain, but it seems to be associated with the disruption of oxidative phosphorylation within individual cells and the alteration of cell membrane permeability (Puritch 1975). One additional favorable property of potassium oleate for use on the balsam woolly aphid is that it causes the loss of the hydrophobic properties of the wax secretions (wool) on the insects body, thereby allowing better contact with the insect. The limiting factors in its use are the mode of application, which is similar to the above, and its lack of residual activity.

Potassium oleate (Insecticidal Soap) is currently being used in Great Smoky Mountains National Park around the Clingmans Dome parking lot and paved trail to the observation tower. The U. S. Forest Service is also experimenting with the use of potassium oleate on Roan Mountain, but lindane continues to be the primary insecticide (P. Berry, USFS-Asheville, personal communication). Silvicultural treatments used to control aphid populations have included complete removal of host trees and species conversion to something other than fir (Hall and Richardson 1973). Lambert and Ciesla (1967) determined that cutting infested trees increased the spread of aphids. This increase was due to the agitation within the stand which caused the release of more aphids than normal into wind currents. The removal of spot infestations is ineffective in slowing aphid spread.

II. INSECT-PLANT INTERACTIONS

In general, phytophagus insects do not exhibit much variation in the basic qualitative requirements for nutrients and plants do not differ much in their qualitative content of required insect nutrients (Dadd 1973; Beck and Reese 1977). The wide range of insect-plant specificity found in nature is associated with the broad category of secondary plant substances or allelochemics (Fraenkel 1959; Fraenkel 1969; Whittaker 1970; Whittaker and Feeny 1971). These compounds influence the developmental physiology of insects by providing terpene and sterol compounds, which insects cannot synthesize, but need for production of juvenile hormones and molting hormones (ecdysones), respectively (Beck and Reese 1977 and references therein). Secondary plant substances are also responsible for other allelochemic interactions such as attractants, repellents, deterrents, inhibitors, and toxicants (Fraenkel 1969; Chapman 1974; Hedin et al. 1974; Hendry et al. 1975).

Insects which feed on plant sap secrete saliva containing an assortment of enzymes and metabolites. Miles (1968a) reported the occurrence of cellulases, polyphenoloxidase, pectin polygalacturonase, other pectinases, several amino acids, as well as indole-3-acetic acid and gibberellic acids. Insects which feed only on phloem sap secrete saliva only during times when they are not feeding, mainly during stylet penetration. However, parenchyma feeders, like the balsam woolly aphid, probably inject saliva continually (Auclair 1963).

The secreted enzymes are associated with stylet penetration, formation of the stylet sheath, and they help break down macromolecules to facilitate nutrient uptake by the insect. Secretion of plant growth substances are more common in parenchyma feeders but the purpose is uncertain. However, within specific concentrations they may enhance the accumulation of nutrients at the feeding site. They are also involved in gall formation associated with cecidogenic insects (Miles 1968b) and probably are responsible for the hypertrophy observed at balsam woolly aphid feeding sites (Balch et al. 1964).

An infestation of phloem feeding aphids, Myzus persicae Sulz., on radish seedlings increased the amount of growth inhibiting substances and decreased the amount of auxin, gibberellins and cytokinins. This in turn resulted in reduced growth, a decrease in the volume of bleeding sap, and wilting (Hussain et al. 1973). The source and physiological causes of these changes in plant hormone concentrations were not investigated.

The effect on wood formation due to aphids feeding on leaves of sycamore maple (Acer pseudoplatanus L.) and lime (Tilia x vulgaris Hayne) were investigated by Dixon (1971a, 1971b). The sycamore aphid caused a reduction in leaf size to a greater extent than would be expected by removal of nutrients by the aphid. This in turn resulted in lowered production of wood during that growing season; without aphids, sycamore maples could produce up to 280 percent more wood. Infestations of the lime aphid caused reduced root growth, but did not affect above ground growth of stems and leaves during the growing

season of the infestation. The following year the leaves of infested trees were smaller than those of uninfested trees. However, the smaller leaves had higher net productivity than larger, uninfested leaves and consequently there was no difference in wood production. These differences between the reaction of the two host species were attributed to the timing of aphid infestations in relation to the physiological and developmental condition of the respective hosts. In both cases reductions in growth were attributed to disruptions in the normal hormonal balance of the host due to the salivary secretions of the insects; however, these secretions were not measured.

Balsam Woolly Aphid-Fir Interaction

Feeding balsam woolly aphids initiate anatomical and biochemical changes within the host that produce physiological modifications resulting in death of the tree. The anatomical changes have been documented, but the biochemical mechanisms remain less clear. Lack of a culture technique for the insect in the laboratory has delayed analysis of the salivary secretions injected into the tree. Determination of the chemistry of these secretions is necessary for an understanding of the biochemical basis of damage to the tree.

The reaction of North American Abies to balsam woolly aphid feeding is manifest through microscopic symptoms, which deal with the response of cells near the feeding sites, and the macroscopic symptoms including growth and form changes.

Microscopic symptoms. Damage to cells is primarily due to chemically induced injury resulting from salivary secretions by the

aphid prior to and during feeding. The saliva produces abnormal cell development around the stylet through either an enzymatic or synergistic action with growth hormones and inhibitors secreted by the aphid or already present in the host tree (Balch et al. 1964). Cortical parenchyma cells are enlarged six or seven times normal, cell walls are thickened, and the cell nuclei become larger. This process is closely followed by hyperplasia in the surrounding parenchyma and sometimes the formation of a secondary phellogen, which is the initial stage in the wound-healing process (Balch 1952; Saigo 1976). Not all Abies in North America are able to complete this process, whereas European silver fir, which has evolved with the aphid, are able to effectively seal off the feeding area before the tree suffers irreversible damage (Kloft 1957). Observations within a European silver fir plantation on Mount Mitchell over a 7 year period revealed that a heavy infestation which developed in 1964 and 1965 rapidly declined over the following two years. Additionally, there was no mortality or apparent tree damage associated with these high insect levels (Amman and Fedde 1971).

Diffusion of balsam woolly aphid secretions results in cellular changes within the phloem, vascular cambium and xylem. The phloem on infested grand fir contained more tangential bands of pholem parenchyma strands and fiber sclereids, had a high number of traumatic resin ducts, and shorter sieve cells than uninfested trees (Saigo 1969). The vascular cambium of infested grand fir contained a wider radial file of cells and increased periclinal and anticlinal division

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of fusiform initials than infested trees (Smith 1967). The cellular
characteristics displayed by the xylem are similar to those of
compression wood: short, thick, highly lignified, reddish tracheids;
an increase in the number of rays; checks in the secondary cell walls
at a larger angle to the longitudinal axis than normal; and a large
reduction in the lumen (Balch 1952; Doerksen and Mitchell 1965;
Foulger 1968). European silver fir growing in Scotland did not
exhibit these symptoms (Varty 1956).

Balch (1952) and Mitchell (1967) observed that the movement of
aqueous solutions of dye through the wood of infested trees was
restricted to fewer annual rings and transported slower than in normal
wood. Puritch (1971) quantified these differences and found that
balsam woolly aphid infestations reduced the permeability of sapwood
by 95 percent; a value which approximated the permeability of
heartwood. Although some tracheids were aspirated, the main cause of
decreased permeability was a reduction in the number of conducting
bordered-pit pairs due to encrusting of the perforations on bordered-
pit membranes of the sapwood; a condition similar to that of heartwood
(Puritch and Johnson 1971; Puritch and Petty 1971). Additionally, the
phenolic composition of balsam woolly aphid affected sapwood was
similar to normal heartwood (Puritch 1977). Thus, balsam woolly aphid
infestations caused the formation of premature heartwood which
resulted in greatly reduced translocation of water and minerals to the
crown. This in turn caused water stress and eventual death of the
tree due to reduction of photosynthesis to near zero (Puritch 1973).

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Macroscopic symptoms. Two types of macroscopic changes are associated with balsam woolly aphid attack, those due to the infestations being concentrated in the crown or those on the central stem of the host tree. Crown infestations exhibit the following sequence of external symptoms: (1) swelling at the nodes, (2) shoots becoming thickened and irregularly twisted and often turning downward, (3) tip inhibition, (4) defoliation, and (5) die-back (Balch 1952).

The loss of height growth combined with the slight increase in diameter growth associated with light to moderate infestations produces extreme stem taper. Crown infestations can cause death of the host after 10 to 20 years of aphid activity. Recovery and resumption of normal growth have been reported for balsam fir in Newfoundland (Schooley 1976).

Macroscopic symptoms of stem infestations are not outwardly apparent prior to the death of the tree. Characteristics of stem infestations include a brief period of increased diameter growth followed by 1 or 2 years of reduced diameter growth. The tree dies rapidly as manifested by the gradual change in foliage color from healthy blue-green, to a faded yellow-green, to a bright rusty-red, and finally to dead-brown. The aphid infestation dies prior to the completion of foliage color change (Amman 1970). This sequence is influenced by the amount and frequency of rainfall during the growing season. Summers with low rainfall or extended dry periods (2-3 weeks) produce the above sequence. However, during summers of adequate rainfall, many aphid infested Fraser fir do not exhibit the final

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color changes to rusty-red and brown. Instead the needles stop at yellow-green and fall off throughout the summer.

Reactions of North American *Abies.* Balsam fir suffers both types of attack, with crown infestations occurring in the maritime zones and stem infestations occurring further inland. This variation is apparently due to differences in climatic factors as opposed to insect or host tree factors (Greenbank 1970).

Bracted balsam fir (*Abies balsamea* var *phanerolepis* Fern.), indigenous to Virginia, usually experiences crown infestations; whereas stem infestations are associated with Fraser fir in the Southern Appalachians (Amman and Talierico 1967).

In the Pacific Northwest, firs exhibit marked differences in their reaction to aphid attack. Grand fir is the most resistant species and can survive stem infestations for 15 years or more. Crown infestations are more common on Pacific silver fir (*Abies amabilis* (Dougl.) Forbes), but stem infestations may also occur. The degree of susceptibility exhibited by Pacific silver fir is related to site quality; trees on poor sites are more tolerant to aphid attack than trees growing on good sites. Subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) is the most susceptible species in the Pacific Northwest, with stem infestations causing tree mortality. However, only subalpine fir growing at the lower limit of its elevational distribution are attacked by the balsam woolly aphid; climatic factors control the insect at higher elevations. Other species of *Abies* native to the Pacific Northwest have not been attacked by the balsam woolly aphid

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within their natural ranges (Mitchell 1966; Harris 1973).

In all regions of North America young fir saplings and seedlings exhibit signs of gouting common to crown infestations. Appreciable mortality occurs only under heavy overstory infestations and recovery of young trees is more common than among mature individuals (Schooley 1976).

Plant Defense Mechanisms

Periderm development and function. Outer bark cells in plants capable of secondary growth provide protection to the plant from the fluctuating environment, prevents desiccation, and loss of metabolites. During the first year of growth this protection is provided by the epidermis, usually only a single layer of cells in thickness. During the second or third growing season, this protective function is provided by the periderm, which develops internal to the epidermis and is composed of three tissue types. The phellogen or cork cambium, is meristematic and produces new cells by periclinal and anticlinal division to accommodate circumference growth. Cells produced external to the phellogen make-up the phellem, or cork. These cells develop suberin, a substance impervious to water, and they die at maturation, providing the required protection to the tree. Cells produced internal to the phellogen form the phelloderm, a tissue consisting of parenchyama cells.

The following terms have been used in the literature to describe periderm associated with various modes of initiation. "First

periderm" has been used for replacement of the epidermis which, in turn, is replaced by the "sequent periderm" (Esau 1977; Srivastava 1964). Periderm developing in response to wounding, either due to mechanical injury or attack by microbes, insects or parasitic plants, has been called "wound" or "pathological periderm" (Bloch 1952; Lipetz 1970). "Secondary protective layer" or simply "periderm" have been used for that formed at abscission scars or resin blisters (Esau 1977; Kozlowski 1973).

Mullick (1969a, 1969b, 1971) and Mullick and Jensen (1973a, 1973b), utilizing cryofixation microtechniques to provide in situ observation of the natural pigmentation with fluorescence and other optical microscopy techniques as well as analysis of compounds responsible for the pigmentation, determined that sequent, wound, and secondary protective layer periderm were identical. However, they differed from first periderm. Based on these observations, Mullick and Jensen (1973) proposed that only two types of periderm be recognized: exophylactic periderm for that which develops internal to the epidermis during the first or second year of growth (formerly first periderm) and necrophylactic periderm (NP) for all other types. In all cases, NP was formed adjacent to dead or non-functional bark tissue and provided protection to the living bark, either from toxic breakdown products associated with cell death or from the external environment, both biotic and abiotic components.

Attempting to identify the cause of NP initiation, Mullick (1975) discovered an impervious tissue which formed prior to NP

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development. This tissue, called non-suberized impervious tissue (NIT), developed prior to formation of necrophylactic periderm and it has been found at all sites associated with NP development.

Therefore, the developmental sequence of tissue in the outer protection bark layer is: epidermis, exophylactic periderm, non-suberized impervious tissue, and necrophylactic periderm. The mechanism responsible for NIT initiation and subsequent NP formation is the development of non-functional phellogen (Mullick 1977). Once the phellogen becomes non-functional, the autonomous, non-specific process of phellogen restoration begins. This involves the dedifferentiation of living cells internal to the non-functional phellogen and redifferentiation into a new cell type. This process is based on the totipotency of all living cells. Dedifferentiation occurs in cells in which NIT forms and again during NP development in cells internal to NIT.

Causes of non-functional phellogen include: 1) response to internal physiological stimuli, as in the sequence of exophylactic periderm to necrophylactic periderm, or 2) due to damage of living cells in already existing NP. Damage can be in the form of mechanical injury, disease, or insect attack, e.g., insertion of a balsam woolly aphid stylet.

Bark morphological features and periderm development. During the time that the exophylactic periderm is functional, the surface of the bark remains smooth and tight. Development of necrophylactic periderm occurs slightly deeper within the bark than the location of the

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exophylactic periderm. The cells external to the newly formed NP are sealed off from the water and metabolites provided by the tree. Consequently, they die and form rhytidome. The bark surface color will change and become flaky as the dead outer layers peel away. After several cycles of NP establishment at successively deeper layers, the bark surface becomes rough and irregular, and eventually develops vertical furrows as the dead layers are sloughed off (Esau 1977).

Rates of NIT formation. The rate of NIT development following mechanical wounding of three species of western conifers was investigated by Mullick and Jensen (1976). Non-suberized impervious tissue formation occurred within 14 days for wounds made in early summer, within 35 days for wounds made in early September, and over 200 days for wounds made in early November. These rates correspond to the annual physiological cycle of plant growth in temperate regions; rates were fastest during the period of high metabolic activity and slowed to a virtual halt during winter dormancy.

The influence of environmental factors on the rate of NIT formation following wounding was studied by Puritch and Mullick (1975). Various levels of water stress were imposed upon potted grand fir seedlings which had been mechanically wounded. Development of NIT slowed after 15 to 20 bars of water stress was achieved and inhibition was proportional to the amount of stress beyond this point. Thus, the ability of a tree to develop adequate protection after wounding was

dependent on physiological and environmental conditions present following wounding.

III. SOUTHERN APPALACHIAN SPRUCE-FIR FORESTS

Fraser fir is endemic to the Southern Appalachians and, in association with red spruce (*Picea* *rubens* Sarg.), occurs in seven disjunct locations in eastern Tennessee, western North Carolina, and southwestern Virginia (Figure 2-1). In the Southern Appalachians, spruce-fir forests are found on mountain summits with elevations above 1750 meters.

Environmental characteristics associated with these habitats are short, cool growing seasons, cold winters, considerable precipitation evenly distributed throughout the year, and high wind velocities. Substantial moisture is also provided by cloud moisture condensation and cloud droplet impaction on vegetation. Soils are generally shallow with a comparatively deep organic layer over sandy-textured A and B horizons; their base saturation values and pH are low (McCracken et al. 1962; Wolfe 1967).

In mature communities of the Great Smoky Mountains, red spruce is dominant in the canopy at elevations between 1370 and 1700 meters, with fir restricted to intermediate and occasionally co-dominant crown classes. Between 1700 and 1830 meters, this mixture becomes more nearly equal, and spruce decreases in importance in size and stem frequency toward the upper limits of this elevational segment. Above 1830 meters, Fraser fir occurs in virtually pure stands (Cain 1935;
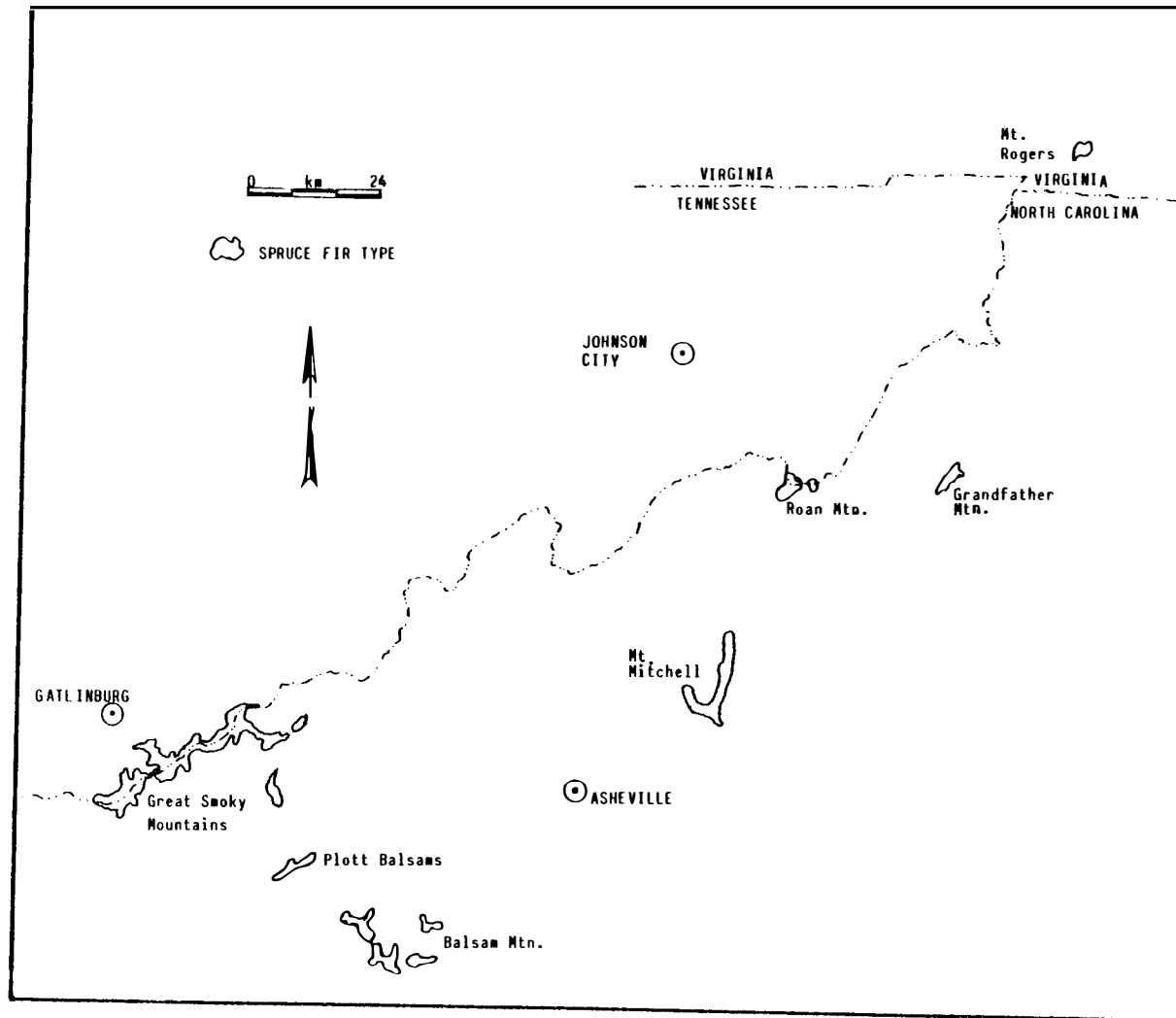
Figure 2-1. Location of red spruce-Fraser fir forest type in the Southern Appalachian Mountains.

Whittaker 1956). Red spruce is locally dominant above 1830 meters on mountains with summits above 1950 meters but only in protected draws. The upper slopes and summits of these higher mountains have pure, even-aged stands of Fraser fir.

Red spruce attains larger size and lives longer than Fraser fir, but Fraser fir grows more rapidly and produces more prolific seed crops. Fraser firs seldom live longer than 150 years and attain a maximum height of 25 meters and diameter of 50 centimeters. Oosting and Billings (1951) found four times as many Fraser fir as red spruce seedlings per 100 square meters. Both species are extremely shade tolerant, and they are capable of resuming normal growth after 50 years of suppression.

Fraser fir serves a dual role in the regeneration of the forests at elevations above 1700 meters. First due to its capabilities as a highly shade tolerant species, Fraser fir seedlings and saplings often form a second generation under the existing canopy. When an opening is created in the canopy, fir reproduction will respond and grow into the canopy. The second role involves Fraser fir as a pioneer species. When openings are created in the overstory and advanced regeneration is not present, Fraser fir will eventually occupy these openings with dense, vigorous reproduction.

The structure and dynamics of the pure fir stands on the mountain summits above 1830 meters are greatly influenced by the climatic and edaphic factors described above. Shallow, coarse-grained, saturated soils combined with exceedingly high winds produce cyclic disturbance

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from windthrow. Fraser fir rapidly replaces itself in these habitats, producing dense, pure, even-aged stands. These stands were found to be less susceptible to balsam woolly aphid infestation than mixed, all-aged, mature spruce-fir stands (Eagar 1978).

CHAPTER 3

STUDY SITES AND METHODS

I. BARK MORPHOLOGY AND APHID FEEDING SITES

Sampling Rationale and Site Selection

Investigations of the relationship of bark morphological features
to balsam woolly aphid feeding site selection were conducted during
summer, 1979. Prior to data collection, a field reconnaissance
determined the range of bark surface morphologies and the associated
tree age, size, and growing conditions. Observations were made along
individual stems as well as among individuals growing under different
levels of competition as indicated by stand density and structure.
During this reconnaissance, developmental patterns of the major bark
morphological features--bark color, texture and lenticel density were
categorized. In addition to the variation associated with tree age,
the amount of crown competition appeared to have the most influence on
differences in bark morphology. This factor, plus the relationship
between balsam woolly aphid infestations and stand structure (Eagar
1978), were used to determine the sampling scheme.

An additional constraint on sample tree selection within a stand
was the desire to focus efforts on younger trees ($\leq$ 20 cm diameter
breast height). Given the extent of aphid-caused mortality and the
inevitability of probable death of all mature Fraser fir in the Great
Smoky Mountains, current research should be applicable to anticipated
future stand structure. Without the discovery of a more practical

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control method than currently exists, it seems likely that future aphid-Fraser fir interactions will occur on trees that are 20 to 60 years old. National Park Service management objectives further restricted research to areas useful in developing resource management strategies and practices, particularly with respect to possible stand manipulation methods.

Future Fraser fir growing conditions can be projected to fit into three general categories representing a gradient of inter-and intraspecific competition. First, stands originally dominated by Fraser fir and having abundant fir seedling cover and/or seed in and on the forest floor at the time of overstory death will develop into dense, pure, even-aged fir stands. This stand structure type is designated Fraser Fir Dominated. Stands meeting this post-aphid condition and chosen as study sites were found on Big Cataloochee, Big Butt, Mount Sequoyah and Clingmans Dome. The second growing condition is that associated with mature spruce-fir forests in which individual and small clusters of fir will be growing in small openings (canopy gaps) caused by mortality of large fir trees. This stand structure type is designated Old-Growth, Spruce Dominated and stands on Mount Collins, Mount Chapman, and Eagle Rocks representing this condition were selected. The final anticipated condition includes Fraser fir dominated stands without abundant seedlings or seeds, large canopy openings in mature spruce-fir stands in which blackberry dominates for a short time followed by light fir recovery (Boner 1977), and fir establishment in high elevation open area (e.g., old burn scars,

balds, blowdowns, and road cuts). In all of these cases, Fraser fir
will be growing under very low stocking densities, essentially as
open-grown trees. This condition is designated Open-Grown Type and
trees fitting this condition were growing on Andrews Bald and on Mount
LeConte between Cliff Top and High Top. Figure 3-1 shows the location
of most stands in which sampling was conducted. All trees sampled
were receiving full sunlight and they had good annual shoot elongation
and crown vigor.

Data and Sampling

Stand density and basal area of the Fraser Fir and the Old-
Growth, Spruce Dominated stands were determined by randomly placed 10
meter square plots within each stand sampled. Three plots were used
in the Fraser Fir Dominated stand and five were used on Mount Collins
in the Old-Growth, Spruce Dominated stand. Plot data were not
collected at the Mount Chapman and Eagle Rocks sites. Diameter breast
height (DBH) and species of all trees were recorded. Site
characterization included elevation, percent slope, and aspect.

Bark surface morphology was evaluated on eight trees per stand in
Fraser Fir Dominated stands. Thirty-two trees were sampled in the
Old-Growth Dominated stands; twenty trees were evaluated on Mount
Collins and six trees on both Eagle Rocks and Mount Chapman. Sixteen
trees were sampled in the Open-Grown Type; eight trees on Andrews Bald
and eight on Mount LeConte. Additionally, individual trees not part
of a particular stand condition were sampled from throughout the
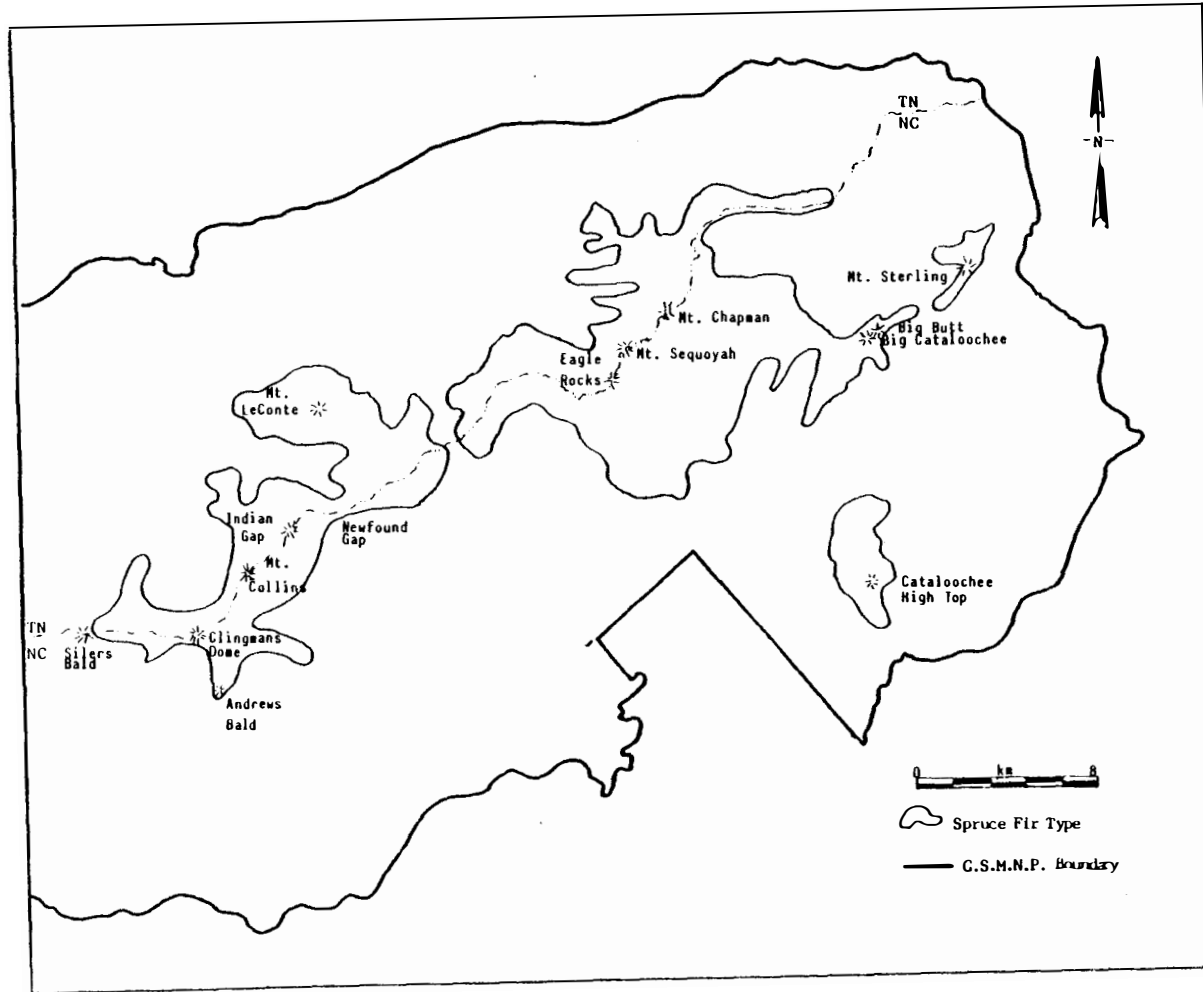
Figure 3-1. Red spruce-Fraser fir forest type in Great Smoky Mountains National Park and location of stands used in this study.

spruce-fir type in the Smokies for evaluation of balsam woolly aphid feeding site preference. Height, DBH, and the percent of the stem occupied by the crown were recorded for each individual.

Evaluations of bark morphology were made at three stem positions: (1) the lower portion of the stem, (2) just above the base of the live crown, and (3) the upper crown. In trees with live crown ratios approaching 100 percent, the second sample was taken at the stem mid-point. The following bark morphological features were evaluated at each stem position.

1. A bark color code was assigned, based on the developmental sequence of gray-green (Munsell 2.5GY6 or 7/2), gray (5Y5 or 6/2), orangish-red (2.5YR5/6 or 8 and 2.5YR4/8), or brown (2.5YR3/4 or 6 and 5YR4/4 or 6).

2. The degree of bark lenticel development was recorded as being light, medium, or heavy.

3. Bark texture was determined as:

    a. tight, smooth bark,

    b. initial splitting of the bark surfac over a small portion of the stem circumference,

    c. shreddy-flaky bark over the entire stem circumference,

    d. rough bark that was not shreddy-flaky, and

    e. vertical furrows beginning to form with the bark surface having a plate-like texture.

Bark texture and lenticel classes are illustrated in Figure 3-2.

Balsam woolly aphid population levels were assigned at each of the sampled stem positions based on the maximum density of insects observed within a 2.54 x 2.54 centimeter square. The following categories were used:

1. No aphids,

2. Less than four wool masses per square,

3. Four wool masses per square to 25 percent covered, and

4. More than 25 percent covered.

Stem diameter and age were recorded at each sampling position and the mean annual growth rate $(cm^2 yr^{-1})$ was calculated from two increment cores per sample position or from stem disks. Bark samples representative of morphological features present within each stand were collected for histological analyses. Pieces of bark approximately one square centimeter in size were removed to the depth of the cambium, placed in 2 1/2 percent buffered glutaraldehyde killing:fixing solution and transported to the lab. Sample preparation procedures at the laboratory are described in the following section.

Data were analyzed on an IBM 370/3031 computer using chi-square test of independence, canonical correlation analysis, and standard correlation procedures of Statistical Analysis Systems programs (SAS 1979).

Figure 3-2. Examples of bark texture and lenticel classes.
(A) Tight, smooth bark with low lenticel density; (B) Tight, smooth
bark with medium lenticel density; (C) Initial splitting of the bark,
usually only over a portion of the stem circumference, and medium
lenticel density; (D) Shreddy-flaky bark with medium lenticel
density; (E) Rough bark, but not shreddy-flaky or furrowed, and with
high lenticel density (white spots are balsam woolly aphids); and
(F) Bark just beginning to develop vertical furrows but it has not
developed a plate-like texture.

A

D
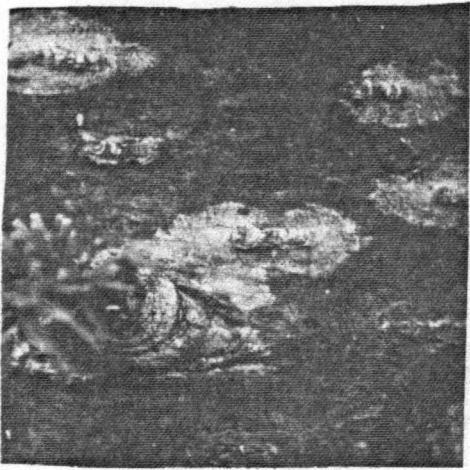
B
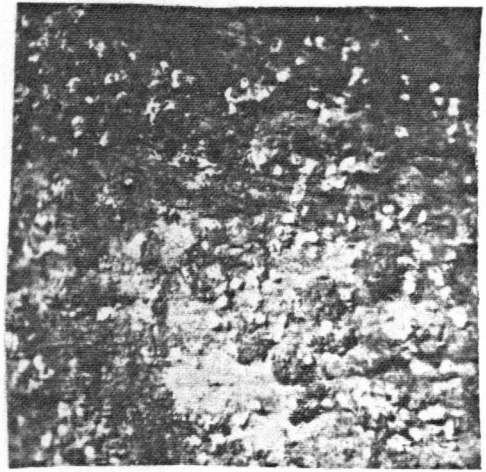
E
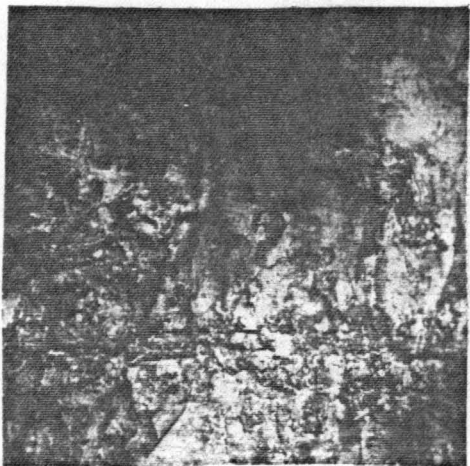
C

F

Figure 3-2

II. WOUND HEALING RESPONSE OF FRASER FIR

The rate of non-suberized impervious tissue (NIT) formation in
Fraser fir was compared along an elevational gradient, between open-
and forest-grown trees, and between presence or absence of balsam
woolly aphids. Sample trees were near Newfound Gap (1550 m), on Mount
Collins (1790 m) and on Clingmans Dome (1950 m). Wounds one
millimeter deep were made on June 19, 1980, with a one millimeter
diameter drill bit. Non-suberized impervious tissue formation was
determined using one primary tree, with confirmation from two
additional trees once a critical time was reached. Two samples per
tree were collected at three day intervals, beginning seven days after
wounding.

Laboratory evaluations of NIT development were made using two
methods--a different method on each bark sample. In the first method,
a square centimeter of bark surrounding the wound was removed, wrapped
in aluminum foil, chilled in ice and returned to the laboratory.
Formation of NIT around the wound was detected using the ferric
chloride-potassium ferricyanide test (F-F test) (Barton et al. 1952 as
adapted by Mullick 1975). Samples were placed in aqueous solutions of
2 percent $FeCl_2$ followed by 4 percent $K_3Fe(CN)_6$. This test is based
on the radial diffusion of ferric ions through the bark tissue.
Within the tissue, the ferric ion is reduced to the ferrous ion by
reaction primarily with phenolic hydroxyls. A water-insoluble complex
is formed when the ferrous ion reacts with the ferricyanide ion.

These solutions stained the tissue a bright blue throughout the zone of permeation, but they do not pass through completed NIT.

Prior to dyeing, smoking hot paraffin was applied to the outer edge of the bark sample to prevent lateral diffusion from the sides or over the top surface. Samples were placed in a Petri dish on a small sponge (2.5 cm long, 2.5 cm wide, 0.5 cm deep). The ferric chloride solution was slowly added until the level was slightly below the upper surface of the sponge. The covered Petri dish was placed in the dark for 24 hours. The bottom of the sample (cambium side) was then rinsed with distilled water, placed on a fresh sponge in a clean Petri dish, and soaked in the potassium ferricyanide solution using the same procedures. Each sample was then rinsed, dried with a paper towel, and carefully cut through the wound with a razor blade. Examination of the depth of penetration of the dye was made with a dissecting microscope.

Prior to NIT formation, the entire bark sample was dyed blue from the cambium to the phellem. Once NIT was completely formed around the wound, the dye could not penetrate into the sealed-off wound area, resulting in a cone of undyed tissue (in transverse or radial sections the undyed area was semi-circular or lens shaped).

On occasion, an extra bark sample was collected and dyeing was accomplished through the wound surface instead of through the cambium. A well was formed around the wound using smoking hot paraffin and staining solutions were applied within the well using a Pasteur pipet. Bark samples were again placed on a sponge in a covered Petri dish;

distilled water was added to the Petri dish to prevent desiccation of the tissue. The remaining procedures and evaluation were the same as for those stained through the cambium.

The second method involved histological techniques to observe cellular and tissue changes within the bark in greater detail. A piece of bark with the wound in the middle was removed and placed immediately into 2 1/2 percent buffered glutaraldehyde killing:fixing solution. In the laboratory, samples were carefully trimmed to a size suitable for sectioning. Air was evacuated. They were dehydrated in a graded series of ethyl alcohol and tertiary butyl alcohol, cleared with cedarwood oil, and embedded in 56.5° C TissuePrep. Samples were softened by soaking the exposed base in 10 percent glycerin. Serial sections 15 micrometers thick were cut on a rotary microtome and stained with safranin, aniline blue, and orange-G. Observations and photographs were made through a Wild M20-EB microscope.

III. EFFECT OF PLANT GROWTH SUBSTANCES ON WOUND HEALING

The influence of selected plant growth substances on the rate of NIT formation was determined using young trees growing in patch-openings within the mature spruce-fir stand on Mount Collins. Plant growth substance used were the auxins indole-3-acetic acid (IAA) and napthaleneacetic acid (NAA); the naturally occurring cytokinin, isopentenyl adenine (2iP); and gibberellin (GA_3). Concentrations used for all four treatments were 10^{-3}, 10^{-6}, 10^{-9}, and 10^{-12} molarity. Wounding and application of the hormones were accomplished

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simultaneously with a Becton-Dickinson Plastipak syringe (1cc) with a 26G 3/8 interdermal bevel needle (outside diameter = 0.46 mm). The bark was penetrated approximately one millimeter and slow, steady pressure was applied until a drop of solution was forced from the point of entry. Two control treatments were used: 1) wounding and simultaneous injection of distilled water and 2) a needle wound without any fluid injected.

In an additional test, indole-3-acetic acid at 10^{-3} and 10^{-6} molarity was applied to each wound three times at three day intervals. Care was taken not to penetrate the bark further than the initial entry. Determinations of NIT formation for all plant growth substance tests were made using the F-F test.

CHAPTER 4

RESULTS AND DISCUSSION

I. BARK MORPHOLOGY AND BALSAM WOOLLY APHID

FEEDING SITE PREFERENCE

Relationships between balsam woolly aphid population levels and
bark morphology of Fraser fir were separated into four components for
analysis with each step building on the prior one. The stem location
with the highest aphid populations and the associated morphological
features at that position were first determined. The second step
concerned the influence that individual tree growth and age
characteristics had on the development of the morphological features
identified in phase one. The influence that selected stand
competition factors had on bark morphology and tree growth
characteristics was then evaluated. Finally, bark morphological
features and growth characteristics of open- and forest-grown trees
were compared in relation to balsam woolly aphid populations.

Anatomical features within the cortex and periderm associated
with the various morphological features were examined for any
difference that might influence aphid feeding site selection.

Balsam Woolly Aphid Feeding Site Preference

Analysis of balsam woolly aphid feeding site preferences using

bark morphological features concentrated on bark color, degree of lenticel development, and position along the main stem. Stands with active infestations and similar infestation history on Big Cataloochee, Mount Sequoyah, Mount Collins, Andrews Bald, and scattered individual trees along the Appalachian Trail were sampled.

Significant relationships were identified between balsam woolly aphid population levels and bark lenticel density, bark texture, and position along the bole (Chi square, p= 0.01; Table 4-1). Stem position was most important with respect to medium and high balsam woolly aphid population levels; they preferred to feed at the base of the live crown. Bark lenticel development showed the strongest relationship to balsam woolly aphid feeding site selection. Sections of the bole with few lenticels had none or less than four aphids per 2.5 cm^2, whereas bole sections with medium and high lenticel densities had high insect numbers. There was a slight tendency for stems with tight, smooth bark to have fewer balsam woolly aphids than stem sections with roughened texture, but this relationship was not as strong as lenticel development.

The influence of bark lenticel and texture conditions on balsam woolly aphid population levels was most evident at the base of the live crown (Table 4-2). Although sample size was too small for significance testing, the trends are worth noting. All samples with no balsam woolly aphids had low lenticel densities and 90 percent were associated with tight, smooth or initial splitting texture classes. Medium and high aphid numbers were found only on stems with medium and

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Table 4-1. Comparison of balsam woolly aphid population levels with stem position, lenticel density, bark surface texture, and bark color.

| | APHID POPULATION LEVELS | | | |
	None	Low	Medium	High
Number of Samples	60	28	14	20
Stem Position:[a]				
% at DBH	55.0	32.2	14.3	20.0
% at Base of Crown	15.0	46.4	71.4	80.0
% at Top of Crown	30.0	21.4	14.3	0.0
Bark Lenticel Density:[b]				
% Low Density	75.0	53.5	14.3	0.0
% Medium Density	23.3	42.9	78.6	70.0
% High Density	1.7	3.6	7.1	30.0
Bark Texture Conditions:[c]				
% Tight, Smooth	48.3	7.1	7.1	15.0
% Initial Splitting	3.3	28.6	14.3	20.0
% Shreddy-Flaky	41.7	53.6	28.6	40.0
% Rough-No Shreddy	1.7	10.7	42.9	20.0
% Furrowed-Platey	5.0	0.0	7.1	5.0
Bark Color:[d]				
% Gray-Green	40.0	28.6	14.3	30.0
% Gray	8.3	10.7	42.9	15.0
% Ogangish-Red	51.7	57.1	35.7	55.0
% Red-Brown	0.0	3.6	7.1	0.0

[a] Chi Square=36.4, 6 d.f.; $p \geq 0.001$

[b] Chi Square=51.1, 6 d.f.; $p \geq 0.001$

[c] Chi Square=47.8, 12 d.f.; $p \geq 0.001$

[d] Chi Square=18.0, 9 d.f.; $p \geq 0.035$

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Table 4-2. Comparison of balsam woolly aphid population levels
with lenticel density and bark surface texture just
below the base of the live crown. Significance
tests were not conducted due to small sample size.

| | APHID POPULATION LEVELS | | | |
	None	Low	Medium	High
Number of Samples	9	13	10	16
Bark Lenticel Density:				
% Low Density	100.0	30.8	0.0	0.0
% Medium Density	0.0	61.5	90.0	75.0
% High Density	0.0	7.7	10.0	25.5
Bark Texture Condition:				
% Tight or Initial				
Splitting	88.9	30.8	10.0	25.0
% Shreddy-Flaky	11.1	53.8	40.0	43.8
% Rough-No Shreddy	0.0	15.4	40.0	25.0
% Furrowed-Platey	0.0	0.0	10.0	6.2

high lenticel densities. These trends identified at the base of the live crown are similar to results based on samples from all stem positions, but they are stronger.

The relationships identified by the Chi square analyses were supported by the canonical correlation evaluations shown in Table 4-3. The first canonical variate, with a canonical R of 0.83 and accounting for 69 percent of the variation, emphasized all morphological features and showed a moderate direct correlation with aphid population levels (0.537). The second canonical variate isolated lenticel development (canonical R = 0.68 and 46 percent of the variation) and showed a strong direct correlation with aphid populations (0.621). Similar relationships were identified in the correlation matrix (Table 4-4).

Tree Growth Characteristics and Bark Morphology

The results of the canonical correlation analysis contrasting the tree bark morphological variables with measurements of tree growth and age are shown in Table 4-3. The first canonical variate maximized the within-group correlation for bark features, thereby indicating how all three features were influenced by growth characteristics. Bark color and texture were more variable along individual tree stems than from tree to tree. From the top of the bole to the butt, the color changed from gray to orangish-red or brown and the texture became more rough. Trees with low growth vigor, as indicated by annual wood volume increment and percent live-crown, developed orangish-red or brown bark with rough texture at a younger age than more vigorously growing individuals. Bark lecticel development was less variable along the

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Table 4-3. Canonical correlation coefficients for bark morphological characteristics tested against balsam woolly aphid populations and individual tree growth variables.*

Canonical Variate	#1	#2
Canonical R	0.83	0.68
Bark Lenticels	0.736	0.672
Bark Texture	0.858	0.253
Bark Color	0.760	0.560
Age at Sample Point	0.789	0.134
Diameter at Sample Point	0.378	0.423
Growth Rate (Area)	−0.116	0.672
Live Crown Ratio	−0.331	0.455
Tree Height	0.187	−0.026
DBH	−0.095	0.492
Aphid Population Class	0.537	0.621
Stem Sample Position	−0.602	0.217

*Coefficients are associated vertically

Table 4.4. Correlation matrix of bark morphological features, balsam woolly aphid populations and individual tree growth characteristics. (* Indicates significance at p < 0.01.)

	DBH	HGT	PRCR	POSP	AGSP	DISP	GRA	APOP	Bark Lenticel	Bark Texture	Bark Color
DBH	1.00	0.70*	0.06	0.06	0.03	0.62*	0.69*	0.35*	0.27*	-0.09	-0.24*
HGT		1.00	-0.55*	0.04	0.21*	0.41*	0.25*	0.32*	0.18	0.11	0.06
PRCR			1.00	-0.01	-0.28*	0.11	0.41*	0.10	0.03	-0.30*	-0.34*
POSP				1.00	-0.73*	0.60*	-0.20	0.02	-0.34*	-0.51*	-0.53*
AGSP					1.00	0.50*	-0.02	-0.01	0.40*	0.51*	0.59*
DISP						1.00	0.80*	0.32*	0.51*	0.29*	0.14
GRA							1.00	0.37*	0.35*	-0.09	0.29*
APOP								1.00	0.52*	0.24*	0.02
Bark Lenticel									1.00	0.35*	0.14
Bark Texture										1.00	0.65*

DBH = Dimeter breast height
HGT = Tree Height
PRCR = Live crown ratio
POSP = Position of sampling point on stem

AGSP = Stem age at sampling point
DISP = Stem diameter at sampling point
GRA = Stem growth rate (area) at sampling point
APOP = Aphid density at sampling point

main stem, and it was strongly influenced by tree age and vigor. Older, larger trees with vigorous growth rates showed the greatest degree of lenticel development.

The correlation matrix provided insight into determining which factor was most important in aphid feeding site selection: lenticel density, tree age, or growth rate (Table 4-4). Although there was a slight tendency for aphids to be associated with older, vigorous trees, the strongest correlation was between aphid populations and bark lenticels. Therefore, balsam woolly aphids required sufficient modification of the bark surface to become established. This modification was usually accomplished through development of lenticels with bark texture making a secondary contribution. Tree age was important in that trees less than 25 years old, which had maintained rapid growth rates, had tight, smooth bark with few lenticels.

Community Factors and Bark Morphology

The influence of selected community factors, principally basal area and tree density, on bark morphological features development was determined from stands on Big Cataloochee, Big Butt, Mount Sequoyah, Mount Collins and Clingmans Dome. All stands except Mount Collins possessed characteristics previously identified as less susceptible to balasm woolly aphid infestations, i.e., Fraser Fir Dominated stands composed of young stems growing at high stocking densities and with small stem diameters (Eagar 1978). Fraser fir contributed 80 percent of the basal area and 78 percent of the stem density in these stands (Table 4-5). Mount Collins represented the other extreme--a stand

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Table 4-5. Mean total basal area (BA) and total density, plus mean basal area, density, and diameter breast height (DBH) by species for Fraser Fir Dominated stands. (n=12)

Stand Parameter	Absolute Values		Relative Values		Mean DBH (cm)
	BA $(m^2 ha^{-1})$	Density (ha^{-1})	BA	Density	
Total	55.0	5700	100.0	100.0	--
std. err.	2.9	802			
Fraser Fir	44.1	4462	80.2	78.3	10.5
std. err.	2.5	766			0.3
Red Spruce	7.9	663	14.4	11.6	10.7
std. err.	1.9	149			1.0
Yellow Birch	1.8	388	3.3	6.8	7.2
std. err.	0.4	90			0.4
Others[1]	1.2	188	2.1	3.3	8.3
std. err.	0.5	90			0.8

[1]Includes Mountain Ash and Pin Cherry

composed of large, mature red spruce and Fraser fir growing at relatively low stocking density--the Old-Growth, Spruce Dominated stands. In these stands, Fraser fir contributed only 21 percent of the basal area but 72 percent of the stem density, whereas the relative basal area of red spruce was 69 percent and relative density was 23 percent (Table 4-6).

Canonical correlation analyses were used to evaluate the influences of community factors upon bark morphological features (Table 4-7). Two analyses were performed; the first compared bark morphology with only the four community variables and the second compared bark morphology with both community variables and individual tree growth variables associated with each of the stands. Analysis with community variables alone produced one significant canonical variate (canonical R = 0.69) and accounted for 48 percent of the variation. Bark lenticel development was greatest in stands with high basal area, a low percentage of fir, and low stem density; typical characteristics of mature stands with large individuals growing at low stocking levels.

The evaluation of community factors combined with the tree growth variables produced three significant canonical variates (Table 4-7). The first variate emphasized the interaction of all three bark features. Development of bark color, texture, and lenticels was more associated with variation along individual stems than with variation from stand to stand; the larger correlation coefficients for the

Table 4-6. Mean total basal area (BA) and total density, plus mean basal area, density, and diameter breast height (DBH) by species for Old-Growth, Spruce Dominated stands. (n=5)

| Stand Parameter | Absolute Values | | Relative Values | | |
	BA (m^2ha^{-1})	Density (ha^{-1})	BA	Density	Mean DBH (cm)
Total	96.0	2650	100.0	100.0	--
std. err.	9.3	171			
Fraser Fir	20.8	1900	21.5	71.7	10.1
std. err.	2.1	178			0.7
Red Spruce	67.0	600	69.3	22.6	27.0
std. err.	7.1	135			5.1
Yellow Birch	8.8	150	9.2	5.7	20.8
std. err.	3.9	50			8.0

Table 4-7. Canonical correlation coefficients for bark morphological features compared with associated individual tree growth variables, with community factors, and for open-grown vs forest-grown trees. Coefficients are associated vertically.

Canonical Variate	Community Factors with Associated Individual Tree Growth Variables			Community Factors Only	Open-Grown vs Forest-Grown Trees	
	#1	#2	#3	#1	#1	#2
Canonical R	0.89	0.78	0.44	0.69	0.80	0.61
Bark Lenticels	0.62	0.77	0.13	0.94	0.17	0.95
Bark Texture	0.89	−0.13	0.43	0.32	0.80	0.32
Bark Color	0.92	−0.20	−0.35	0.44	0.96	−0.15
Stand Basal Area	0.42	0.66	−0.37	0.96		
% Fir Basal Area	−0.35	−0.63	0.38	−0.88		
Stand Density	−0.34	−0.56	0.09	−0.82		
% Fir Density	−0.34	−0.55	0.06	−0.75		
Aphid Density	0.35	0.43	0.49			
Stem Position	−0.86	0.25	0.21			
Live Crown Ratio	−0.12	0.04	−0.35		−0.51	0.36
Stem Age	0.76	0.09	0.32		0.90	0.18
Stem Diameter	0.71	−0.31	0.15		0.36	0.78
Growth Rate (Area)	0.13	−0.32	0.36		−0.19	0.79
Tree Height	0.07	−0.16	0.31		0.32	0.05
DBH	−0.06	−0.19	0.46		−0.17	0.51
Group (open/forest)					0.45	−0.49

community/tree variables were for tree growth variables and position on the stem.

The second canonical variate isolated bark lenticel development and showed trends similar to the analysis with community factors alone, i.e., more advanced lenticel development occurred in mature stands with large but few trees. In this analysis (Table 4-7), individual tree growth characteristics differed from individual tree characteristics reported in Table 4-3 (page 49). In the previous analysis, which was based on observations made on trees growing both within stands and in open conditions, greater lenticel development was associated with fast growing, large trees having high live-crown ratios. But in this analysis, which only used trees growing in forest conditions, greater lenticel development occurred on slow growing trees with overall small size and there was no correlation with live-crown ratio. These results emphasized the influence of within-stand competition on lenticel development.

Young individual fir trees growing in mature spruce-fir stands had slower growth than those growing in young, even-aged, pure fir stands or those growing in the open. The increased lenticel development was caused either by competition from larger individuals or the concentration of similar number of lenticels over less bark surface area on the slower growing trees. None of the trees sampled in this study were suppressed individuals; all sample trees exhibited moderate or better growth rates determined by terminal and lateral shoot growth, needle color and density, and live-crown ratio.

The third canonical variate (Table 4-7) was also significant and isolated bark texture. There was a slight tendency for rough texture to develop on fir trees growing in very dense, young stands. These trees usually exhibited good vigor. Additional factors, not a part of this study, undoubtedly influenced bark texture. For example, the amount of direct sunlight reaching the main stem could have influence textural development, for conditions identified by this canonical variate indicate low levels of sunlight encourage rough bark texture.

Comparison of Open-Grown and Forest-Grown Trees

Observations in the field indicated a different sequence or rate of development of bark features between open-grown and forest-grown trees. After separating the data as to open-grown or forest-grown, comparisons were made between bark morphological features and tree growth characteristics using canonical correlation analysis (Table 4-7). The first canonical variate focused on forest-grown trees and the second on open-grown trees.

Bark color and texture were more orangish-red and more rough for forest-grown trees than for open-grown trees. Bark lenticel development was greatest on open-grown trees and these trees maintained smooth bark texture with a gray color over a large range of size and age classes.

Bark Anatomical Characteristics and Morphology

Histological examinations were made of cortical tissue associated with various bark surface morphologies in order to determine if

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cortical tissue differences might be responsible for balsam woolly aphid feeding site preference. Comparisons were made between bark samples heavily infested with balsam woolly aphids and samples with similar morphological features but without aphids. Characteristics observed were:

1. abundance and distribution of sclereids,

2. size and location of resin cells,

3. rhytidome thickness,

4. phellogen activity,

5. cortex thickness and cell size within the cortex,

6. occurrence of unusually stained material in cortical or periderm cells, and

7. formation of necrophylactic periderm around balsam woolly aphid feeding sites.

The only variations observed were those of developmental processes associated with growth and aging (Chang 1954; Saigo 1969; Borger 1973; Esau 1977). Samples from young trees had little or no rhytidome, infrequent and small sclereids, few and small resin cells, and cortical thickness was dependent on overall bark thickness. There was a general tendency for resin cells to develop below bark lenticels, but aphids feeding on lenticels did not reach the resin cells. Resin cells also occurred throughout the cortex, independent of surface lenticels.

Unusual cell staining was associated with enlarged cells caused by balsam woolly aphid feeding. This response was similar to that

reported by Balch (1952) for balsam fir growing in eastern Canada. Necrophylactic periderm was not found at aphid feeding sites (Figure 4-1). Bark samples containing balsam woolly aphids were collected late enough in the insect's life cycle (July 10 and September 5) for necrophylactic periderm to have developed. Balsam woolly aphid feeding site selection on fir stems appeared to be influenced by external morphological features and not internal anatomical features.

Summary of Morphological Features

Morphological characteristics of the bark influence balsam woolly aphid population levels between trees and along the bole of individual trees. For successful penetration of the bark and extension of the stylet into the cortical parenchyma cells, the balsam woolly aphid required bark conditions available at lenticels and furrows. A common characteristic of these two features was a non-existent or shallow rhytidome and a roughened, irregular bark surface. Consequently, initial penetration of the surface was facilitated by the reduction in hard, dead tissue thickness and close proximity of cortical parenchyma cells to the surface. The microscopic splitting of the rhytidome at these sites provided an easy point of entry for the aphid's stylet. Tight, smooth bark presented a physical obstruction to stylet penetration. Areas with rougher texture also provide protection from the environment to the aphid (Balch 1952).

As the stem ages, bark morphological features on Fraser fir exhibit the following developmental sequence:

1. Tight, smooth, greenish-gray bark supporting needles

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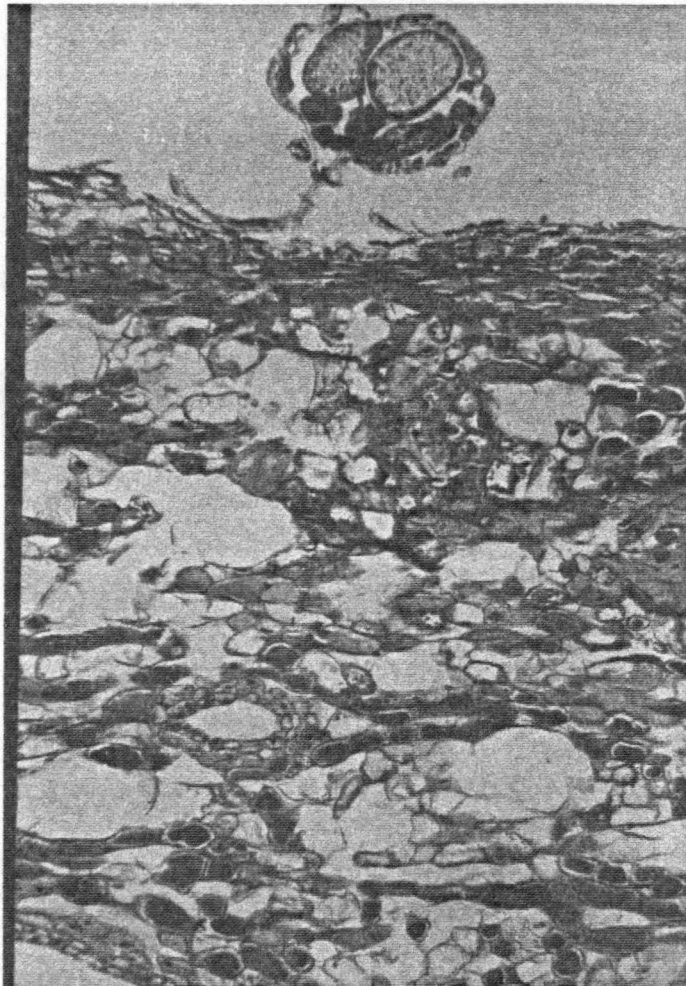
Figure 4-1. Balsam woolly aphid feeding site collected June 30, 1980 near Indian Gap. Stylet can be seen entering bark through an area of light rhytidome development adjacent to a lenticel which is to the left of the area photographed. Pocket of blueish cells directly beneath the insect shows tissue reaction to salivary secretions. Cells have enlarged and have large, distinct nuclei. This isnsect was a mature adult which had overwintered; however, there was no sign of non-suberized impervious tissue or necrophylactic periderm development. x 72

forms the first covering of the main stem.

2. Tight, smooth bark becomes grayer, needles

    abscise, and lenticels develop at the leaf scars.

3. When the bark surface first splits, the color changes

    to gray-brown, and the lenticels continue to develop,

    often forming short horizontal segments.

4. With continued bark splitting and flaking, the bark

    changes color, becoming orangish-red, and the lenticels

    become corky in appearance.

5. Vertical furrows, with plates of dead bark adhering

    on the surface between the furrows, begin to develop

    and the color becomes dull reddish-brown and lenticels

    become less apparent, having been cut off from the

    cortex by bark shedding.

6. Thick, heavy plates only develop on large, old trees.

This sequence occurred within five to ten years on slow-growing trees.
Fast-growing individuals, those without vertical or lateral crown
competition, maintained tight, smooth, gray bark with scattered
lenticels for up to 25 years. Consequently, Fraser fir growing under
optimum conditions lacked morphological features for an extended
period which were necessary for large aphid populations.

The combination of bark morphology and enhanced vigor of fast-
growing firs permitted extended life after balsam woolly aphid
colonization. For example, the last living Fraser firs on Mount
Sterling, the area in the Park with the longest history of aphid

activity, were growing without much competition and had tight, smooth bark until they were 12 meters tall. These trees died in 1979; however, forest-grown firs of similar size in the same area died about 1970.

Two other factors in addition to feeding site availability should be considered with respect to vigorous-growing trees being able to live longer than average after aphid infestation. First, the bark of vigorously growing trees was thicker than that of less vigorous trees, especially during the first third of the trees normal life (Hale (1955) and observations made during this study). Additionally, bark exposed to full sunlight develops deeper periderm and more cork (phellem) than stems protected from the sun (deZeeuw 1941; Mogensen 1968). It is possible that these factors retard damage to the tracheids caused by the salivary secretions of the aphid. Saliva of aphids feeding on stems with thick periderm and cortex has to diffuse through more tissue before it makes contact with the xylem. Substances secreted by the aphid would become more dilute with increased diffusion time. This added contact time with living tissue could also result in metabolic alteration of the compounds secreted by the aphid into less potent or toxic forms.

The second factor involves the volume of sapwood available for translocation of water and minerals. Vigorously growing, younger trees have large annual growth increments and a higher percent of their cross-sectional area is composed of sapwood than in less vigorously growing individuals (Panshin and deZeeuw 1980).

Consequently, the compounds responsible for the reduction in permeability through the tracheids due to balsam woolly aphid salivary secretions (Puritch and Johnson 1971; Puritch and Petty 1971) are further diluted resulting in a lower percentage of damaged tracheids. Damage to the tree on a per insect basis, in terms of the trees ability to deliver water, minerals, and metabolites to the crown, is less for vigorously-growing trees. This, in combination with fewer feeding sites on these trees, enables vigorously-growing trees to survive longer than those experiencing more intense competition.

Distribution of balsam woolly aphids along the bole of maturing firs verified that bark morphology influenced feeding site preferences. The highest concentration of aphids was near the base of the live crown, where bark morphology had progressed to extensive splitting with maximum lenticel development. At positions above this zone, the bark surface resembled that of young, vigorous, fast-growing fir saplings and there were not many feeding aphids. Logically, there should have been more aphids in the top of the crown due to the passive dissemination by wind and subsequent interception in the top of the crown.

Relationships between growth vigor, bark development, tree age and aphid populations become important with respect to the age at which Fraser fir produces cones. Young fir trees established in the blowdown between LeConte Lodge and High Top on Mount LeConte were sampled for age and cone occurrence (Table 4-8). The average age at 25 centimeters above ground of the 15 youngest trees with cones was 21

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Table 4-8. Age and DBH of the 15 youngest trees found with
cones on Mount LeConte during 1979.

DBH (cm)	Age at DBH	Age at Base
15.0	14	35
13.3	12	28
8.0	11	16
9.5	13	18
10.1	15	19
8.2	11	17
12.2	14	22
8.9	13	18
8.6	11	16
14.1	15	27
9.0	15	19
9.6	14	19
14.2	16	29
7.9	12	17
8.9	11	18
Mean 10.5	13	21

years; the youngest was 16 years old. Early cone production can occur when firs have bark morphological characteristics that are not conducive to balsam woolly aphid establishment. The high vigor of these young trees readily supports cone energy requirements.

This relationship provides insight into the future of Fraser fir in the Great Smoky Mountains. As the last of the Fraser fir in the southwest end of Fraser fir's distribution in the park become infested and die over the next five to seven years, firs in the northeast end of the distribution will be reaching sufficient age to support aphids. Prevailing winds will aid the aphid in reinfesting the northeast end. Thus, a cyclic situation will develop: initial infestation followed by limited recovery and regeneration of fir followed by reinfestation, etc. The first cycle, which is almost complete, will take about 30 to 40 years. Subsequent cycles will probably take longer since trees will be smaller and less frequent in the landscape and, therefore, will have a lower probability of intercepting airborne aphids.

II. WOUND HEALING RESPONSE OF FRASER FIR

Formation of non-suberized impervious tissue (NIT) in the cortex of Fraser fir was primarily influenced by the environment and the structure of the stand in which the trees were growing. Differences in the rate of NIT formation and subsequent establishment of necrophylactic periderm (NP) were influenced by elevation, open- and forest-grown conditions, and infested and uninfested trees (Table 4-9). Examples of the F-F test for NIT formation are shown in Figure

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Table 4-9. Rates of non-suberized impervious tissue (NIT) formation after mechanical wounding on June 19, 1980 for open- and forest-grown Fraser fir at three elevations.

Location	Elevation (meters)	Condition	APOP[1]	Days to NIT
Clingmans Dome	1950	Forest[2]	1	26
Clingmans Dome	1950	Forest[2]	1	26
Clingmans Dome	1890	Open	2	20
Mount Collins	1800	Forest[3]	3	20
Mount Collins	1800	Forest[4]	1	23
Mount Collins	1800	Forest[4]	4	20
Mount Collins	1800	Forest[4]	1	23
Mount Collins	1800	Forest[4]	1	23
Mount Collins	1800	Open	1	20
Indian Gap	1600	Forest[4]	4	18
Indian Gap	1600	Open	1	17
Newfound Gap	1550	Open	1	17

[1]Aphid population level, described on page 36.

[2]A typical Fraser Fir Dominated stand.

[3]A typical Old-Growth, Spruce Dominated stand, sample trees growing subordinated to mature red spruce.

[4]A typical Old-Growth, Spruce Dominated stand, sample trees growing in canopy gaps.

4-2. Examples of the histological examination of bark tissue around wounds are shown in Figures 4-3 and 4-4.

Forest-grown trees at high elevation (1950 m) required seven more days for NIT formation than did trees growing at the low elevation (1600 m). Trees at an intermediate elevation (1800 m) required four more days than those at low elevation. Variation in the rate of NIT formation of open-grown firs along the elevation gradient was three days, with firs at high and intermediate elevations requiring 20 days and those at low elevation requiring 17 days after wounding. At the same elevation, open-grown trees produced NIT an average of three days earlier than forest-grown trees at the low and and intermediate elevations and six days earlier at the high elevation site.

Rates of NIT formation appeared to be influenced by micro- and macro-environmental differences associated with stand density and elevation. Conditions for vigorous tree growth, which are directly related to enhanced rates of physiological and biochemical processes, are associated with reduced stand density (Kramer and Kozlowski 1979). Trees lacking lateral competition are able to maintain high live-crown ratios, they receive and utilize more sunlight and they have access to more available moisture and nutrients. In addition, the micro-climate within a closed stand is cooler than in the open, thereby reducing the rate of biochemical processes (Salisbury and Ross 1978) which influences the rate of NIT formation. Studies on the rate of wound periderm formation in potato tubers and sugar beet corms (Artschwager 1927; Artschwager and Starrett 1931, 1933) as well as the rate of
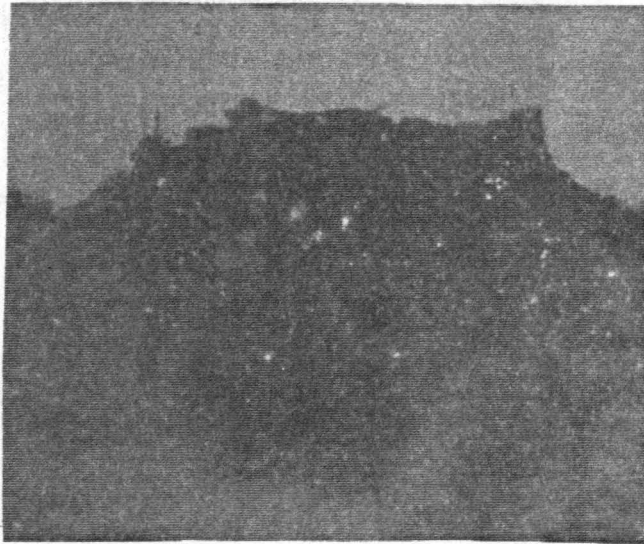
Figure 4-2.   Examples of results of ferric chloride-potassium ferricyanide test for formation of non-suberized impervious tissue. (A) Dye applied through the cambium.   (B) Dye applied through the bark surface.   Samples were from Fraser fir growing on Mount Collins. Injuries were made on June 19, 1980 and collected on July 20, 1980. Non-suberized impervious tissue had formed by July 12, 1980, followed by necrophylactic periderm.   For photograph A, the test solution penetrated the cortex to the base of the necrophylactic periderm, the zone of reddish-brown tissue.   x20
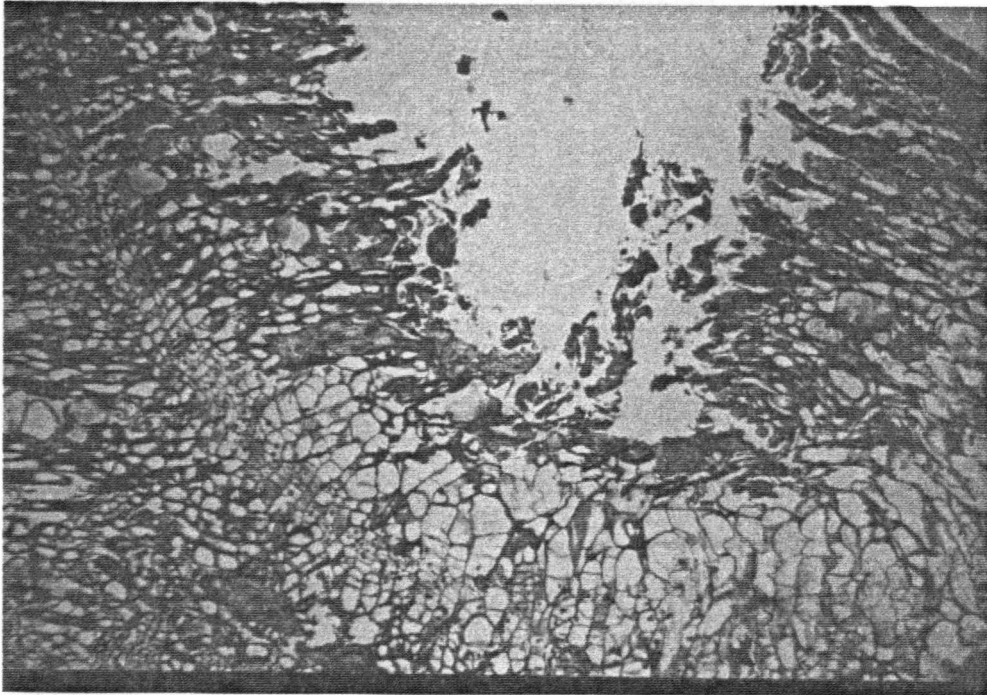
Figure 4-3. Development of non-suberized impervious tissue (NIT)
with partial necrophylactic periderm (NP) around a mechanical wound in
the bark of Fraser fir growing on Clingmans Dome. This sample was
collected on July 15, 1980, following injury on June 19, 1980. The
enlarged, irregularly shaped cells comprise the NIT. Necrophylactic
periderm has formed along the lower left side of the wound below the
enlarged NIT cells; and is detectable by the uniform cell size, radial
arrangement, and conspicuous nuclei. x36.

Figure 4-4. Transverse section through the outer zone of necrotic tissue which developed between the point of wounding and non-suberized impervious tissue (NIT). This sample was from Newfound Gap and formed NIT 17 days after wounding on June, 19, 1980; the shortest time observed at any of the locations used. Necrophylactic periderm can be seen on both sides and in spots along the base. x36.

development of first periderm (exophylactic periderm) in several woody
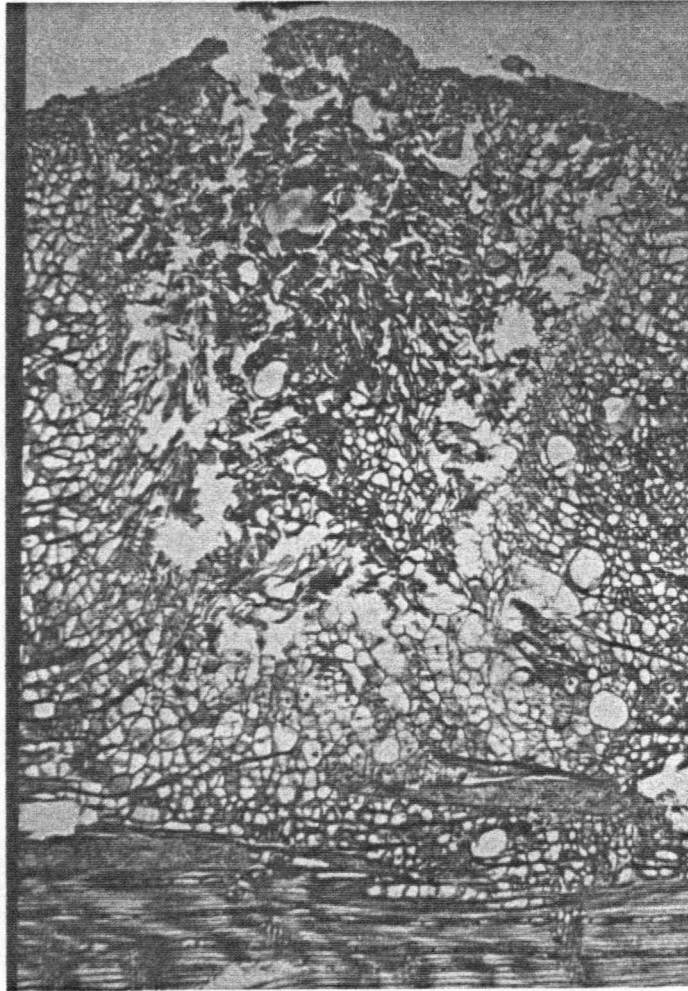
angiosperm seedlings (Borger and Kozlowski 1972) were positively

correlated with temperature. Mullick (1976) found differences of five

days in the rate of NIT formation for the same _Abies_ _amabilis_ wounded

on about the same day for two successive growing seasons. This

difference was attributed to environmental conditions prior to

wounding. Warmer temperatures, more sunny days, and high

precipitation resulted in greater tree vigor the year of the faster

rate of NIT formation.

The differences in the response time of trees to wounding at low

and high elevation sites could have been related to differences in the

beginning of the seasonal growth cycle. Trees at 1600 meters begin

growth earlier in the spring than those at 1950 meters. Thus, the

physiological state, in terms of seasonal allocation of metabolites to

the various growth functions, differed with elevation, perhaps

influencing the rate of NIT formation. However, the date of tree

wounding (June 19) was late enough into the growing season that

differences in physiological condition should not have been great. As

with trees growing in the open versus those in the forest, temperature

differences between the low and high elevation sites were probably the

most important determining factor.

Mechanically wounded, aphid-infested firs on Mount Collins

demonstrated more rapid formation of NIT than uninfested trees growing

within two meters of the infested tree (Table 4-9 page 66). Balsam

woolly aphid-infested firs experience a period of vigorous diameter

growth during the two or three years following colonization.

Substances within the salivary secretions of the aphid are suspected

of inducing this stimulation of cambial activity (Balch 1952). These

substances apparently also stimulate biochemical and physiological

processes within the cortex and periderm, thereby accelerating the

formation of NIT. The vascular cambium and phellogen have similar

meristematic properties.

The enhancement of NIT formation resulting from wounds within the

cortex of aphid-infested trees occurred at positions on the bark not

directly affected by the aphid. Wounds were not made proximal to

aphid feeding sites; rather they were located at least two centimeters

from the insect. Thus, any influence from insect secretions on the

formation of NIT was after diffusion and possible dilution of the

responsible compounds.

III. EFFECT OF PLANT GROWTH SUBSTANCES ON WOUND HEALING

Balch (1952) and Balch et al. (1964) suggested that the auxin,

indole-3-acetic acid (IAA), was responsible for stimulation of cambial

activity as well as the formation of "rotholz," the compression-like

wood formed in aphid-infested trees. Indole-3-acetic acid was

suspected because of its role in formation of normal compression wood

(Wershing and Bailey 1942) and by experiments in which excessive

concentrations of IAA (10 mg IAA in 1 g of lanolin) were applied to

scarified fir bark surfaces (Balch 1952). Results included death of

the shoot in some cases, callous formation at the point of application

in some, and "rotholz" formation in others. The role of NIT and NP in the wound healing process was not known when Balch's experiments were conducted.

To better understand the interaction between the balsam woolly aphid and Fraser fir, and especially the mechanism by which aphid feeding suppresses wound healing in the cortical tissue penetrated by the aphid stylet, experiments were conducted combining mechanical wounding with injection of plant growth substances. Plant hormone concentrations chosen were those within the normal physiological range of forest trees (Kramer and Kozlowski 1979). Results are presented in Table 4-10.

Mechanical wounding combined with a single injection of auxin-like compounds, the naturally occurring IAA and the synthetic naphthaleneacetic acid (NAA), retarded NIT development for 11 additional days compared with just mechanical wounding, i.e., 31 days compared to 20 days. Only 23 days were required for NIT formation after mechanical wounding and injection of distilled water. Response time was identical for seven of the eight auxin treatments. The one exception required an extra two days. Trees receiving the other two plant growth substances, the naturally occurring cytokinin, isopentenyl adenine (2iP), and gibberellin (GA$_3$), required the same number of days to form NIT as the trees receiving the injection of distilled water.

Hormone concentrations did not affect the rate of NIT formation in this study. Indole-3-actetic acid in concentrations ranging from

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Table 4-10. Rates of non-suberized impervious tissue (NIT) formation as influenced by inoculation with selected plant growth substances.[1]

Treatment	Concentration (Molarity)	Days to NIT
IAA	10^{-12}	31
IAA	10^{-9}	31
IAA	10^{-6}	31
IAA	10^{-3}	31
NAA	10^{-12}	31
NAA	10^{-9}	31
NAA	10^{-6}	33
NAA	10^{-3}	31
2iP	10^{-12}	20
2iP	10^{-9}	23
2iP	10^{-6}	23
2iP	10^{-3}	23
GA	10^{-12}	26
GA	10^{-9}	20
GA	10^{-6}	20
GA	10^{-3}	20
wound only	–	20
H_2O	–	23
IAA[2]	10^{-6}	31
IAA[2]	10^{-3}	31

[1]Trees were growing on Mount Collins in full sunlight within canopy gaps in typical Old-Growth, Spruce Dominated stand. Experiments were initiated 29 July 1980.

[2]An open grown tree that received two additional follow-up inoculations of IAA at 3 day intervals.

10^{-12}M to 10^{-3}M produced the same results. Naphthaleneacetic acid showed similar results with one exception. Even treatments of IAA applied to the same wound three times at three day intervals produced NIT after 31 days, eleven days after the control treatment of wounding only. Such treatments, however, probably more nearly simulate balsam woolly aphid feeding than a single hormone treatment.

Although circumstantial, it appears that the balsam woolly aphid is able to retard or substantially inhibit plant defense mechanisms within the periderm of Fraser fir. Normally these trees should be able to form NIT in about 20 days at balsam woolly aphid feeding sites; i.e, about the time that the insect would emerge from diapause and develop into the first instar larvae. Histological examination of aphid feeding sites collected throughout the summer failed to produce a single case of necrophylactic periderm formation in Fraser fir growing in the Great Smoky Mountains. Auxin-like compounds slowdown the rate of NIT and subsequent NP formation around mechanical wounds. Although not determined for the balsam woolly aphid, literature supports the likelihood of auxin-like compounds, or other chemicals that produce responses similar to this plant hormone, in balsam woolly aphid salivary secretions (Balch et al. 1964; Miles and Lloyd 1967; Miles 1968a, 1968b; Schaller 1968 as seen in Miles 1972; Sapio et al. 1982). Furthermore, since the balsam woolly aphid is a parenchyma feeder (Balch 1952) and parenchyma feeders secrete saliva continually (Auclair 1963), a constant supply of the auxin-like compound (or other active compounds) is available to interfere with normal wound healing

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processes. A constant supply, as opposed to the single application used in this study, could provide for a longer inhibition of the wound healing processes.

It is unlikely that the balsam woolly aphid secretes compounds specifically to block or inhibit necrophylactic periderm formation; European silver fir is able to seal off the feeding site within the life of the insect (Kloft 1957). The inability of Fraser fir in the Great Smoky Mountains to form NP allows either the same substances responsible for the inhibition of wound healing, or others in the saliva, to diffuse into the xylem, causing rotholz and the reduction in translocation, and eventually death of the tree.

CHAPTER 5

CONCLUSIONS

1. The amount of lenticel development was the best predictor of
    balsam woolly aphid activity along stems of Fraser fir.

2. Lenticels were more frequent at the base of and just within
    the live crown of trees growing in stands (forests).

3. Bark surface features on open grown trees were not suitable
    for balsam woolly aphid feeding until the trees were about 25
    years old.

4. Vigorous, open-grown Fraser firs produce cones at an average
    age of 21 years. Therefore under ideal conditions, the
    balsam woolly aphid should not kill the next generation of
    Fraser fir until it has had time to produce progeny for a
    third post-aphid generation.

5. The rate of formation of nonsuberized impervious tissue and
    necrophylactic periderm in Fraser fir during the growing
    season was similar to that in western <u>Abies</u>.

6. Factors which affect tree vigor in a positive manner and
    enhance biochemical rates control the rate of nonsuberized
    impervious tissue and necrophylactic periderm formation.
    Trees growing in the open and trees at lower elevations
    produced nonsuberized impervious tissue and necrophylactic
    periderm faster than those growing within a stand or at
    higher elevations.

7. Auxin-like substances retard the formation of nonsuberized
   impervious tissue and necrophylactic periderm around
   mechanical wounds.

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VITA

Christopher Eagar was born in Chattanooga, Tennessee on December 28, 1946. He attended elementary school in that city and was graduated from Notre Dame High School in June 1965. He entered Tennessee Technological University in Cookeville, Tennessee in September 1965 and transferred to the University of Tennessee, Knoxville in September 1967. He received a Bachelor of Science degree in Business Administration (Marketing) in December 1969.

Mr. Eagar was drafted into the United States Army in May 1970 and served until December 1971. After an extended period of traveling throuhout the western United States, he was employed as a Department Manager for Montgomery Ward Company in Fort Collins, Colorado. He returned to the University of Tennessee, Knoxville in March 1975 and began the necessary prerequisite course work for admission into graduate school in Forestry. He was admitted to Graduate School in March 1976 and was awarded a Master of Science degree in Forestry in August 1978.

Mr. Eagar entered the Graduate Program in Ecology at the University of Tennesse, Knoxville in September 1979 and was awarded a Doctor of Philosophy in Ecology in June 1985. In July 1981 he was employed by Uplands Field Research Laboratory, Great Smoky Mountains National Park and since January 1982 he has worked full-time as a research ecologist.

He is married to Anne L. Coker.