

**SEASONAL VARIATION IN CONSTITUTIVE AND INDUCED DEFENSES
OF SPRUCE (*PICEA* SPP.) HOSTS OF THE WHITE PINE WEEVIL
PISSODES STROBI PECK**

by

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ABSTRACT

The white pine weevil, *Pissodes strobi*, is a major pest of regenerating spruce in British Columbia, and the damage caused by weevil attack results in a severe reduction in plantation productivity and wood quality. As a result, the planting of Sitka spruce, except in a small portion of its range, is no longer recommended in B.C. Control attempts have met with little success; however, it is believed that host resistance will be an essential part of an effective Integrated Pest Management plan for the weevil. My objective was to examine the induced resin response and the constitutive resin canal system of Sitka spruce to identify potential resistance mechanisms and to determine how those mechanisms were affected by seasonal growth and development of the tree.

Histological examination of cross-sections of laterals taken at different times during the season were used to compare the extent of components of the constitutive resin canal system between resistant and susceptible families of Sitka spruce. Similar methods were used to compare the capacity of resistant and susceptible trees to produce a traumatic resin response. Weekly measurements of leader apical bud phenology and radial growth were used to determine if differences in leader growth and development existed between resistant and susceptible trees which could affect resin canal density in the leader. Artificial wounding of tree leaders at different times of the season and at different densities of wounding was used to determine if there was a seasonal effect on the production of traumatic resin and if the response level was related to the wounding level.

Compared to susceptible trees, resistant trees tended to have larger inner resin canals and thinner bark, and thus, a greater percentage of bark occupied by inner resin canals. Furthermore, both resistant and susceptible trees showed an increase in resin canal size and a decrease in resin canal density through the season. The level of the traumatic response produced by artificially wounded trees was significantly higher in resistant trees than in susceptible trees and was also found to be highest in early flushing trees. No difference in rate of leader radial growth was observed between resistant and susceptible trees, but buds on resistant trees flushed earlier than those on susceptible trees. The traumatic response was found to be lowest in trees wounded early in the season, and was also found to increase in a density dependent manner with the wounding level.

Although several traits were present to a greater degree in resistant trees than susceptible trees, no single trait was emphasized in every resistant individual, suggesting that effective resistance is based on a combination of traits. Furthermore, the variability of these traits through the season reinforces the importance of host-insect phenology in the success or failure of an attack.

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1. GENERAL INTRODUCTION

1.1 LIFE CYCLE OF THE WHITE PINE WEEVIL

The white pine weevil (=spruce weevil)*, *Pissodes strobi* Peck (Coleoptera: Curculionidae) is a major pest of young spruce (*Picea* spp.) stands throughout British Columbia and North America (Humble *et al.* 1994). On the coast of British Columbia, the spruce weevil's primary host is Sitka spruce (*Picea sitchensis* (Bong.) Carr) and in the interior of B.C., the spruce weevil's principal hosts are Engelmann spruce (*Picea engelmannii* Parry), white spruce (*P. glauca* (Moench) Voss) and their hybrids (*P. engelmannii* x *glauca*) (Kiss and Yanchuk 1991). Host trees up to 15 m tall and between the ages of 4 and 30 are most susceptible to attack. *Pissodes strobi* emerge in early spring after overwintering in the duff on the forest floor or, at some coastal sites, overwintering on the tree; then the weevils fly or walk to nearby host trees, where they feed, mate and lay their eggs (Alfaro *et al.* 1995, Silver 1968). Most feeding occurs on lateral branches; however, oviposition occurs in holes which are excavated just below the apical bud of the tree's terminal leader and then sealed with a fecal plug. Following hatching, the larvae mine downwards in the phloem, eventually forming a feeding ring that girdles the leader as the phloem is consumed. The larvae then pupate in chambers called chip cocoons, which are excavated from the xylem, and covered with wood fibres. The new adults start to emerge from

* Historically in Canada *Pissodes strobi* was classified as three separate species based on host preference. In the west, *Pissodes sitchensis* was found on Sitka spruce and *Pissodes engelmannii* was found on Engelmann spruce, and in the east *Pissodes strobi* was found on eastern white pine. Subsequent studies resulted in the amalgamation of the three species, and although the official common name of *Pissodes strobi* is considered to be white pine weevil in western North America, it is often referred to as the spruce weevil.

late July to September, feeding and dispersing; but, as the temperatures drop and the number of daylight hours decreases, the weevils move to the duff and hibernate.

1.2 DAMAGE

Terminal leader mortality can result in severe growth deformities when the lateral branches compete for apical dominance, forming new leaders which can be attacked in the following year. In areas that are heavily attacked, productivity of young spruce plantations can be severely reduced. In addition, tree defects caused by attack can reduce grade and the amount of lumber that can be taken from a tree. In some spruce plantations that were heavily attacked by weevils, the trees never reached free growing status (Hall 1994). In certain coastal areas that are particularly susceptible to heavy weevil attack, Sitka spruce is not currently being planted because the damage and financial losses are so great (Alfaro 1982, Hall 1994). All of these factors can cause serious financial losses to the B.C. forest industry (Alfaro 1989a). In addition, sustainable ecosystem management and biodiversity guidelines are not being adequately fulfilled by avoiding replanting spruce, so a way must be found to plant Sitka spruce to enable restoration of damaged ecosystems and avoid large financial losses due to weevil attack.

1.3 CONTROL

The spruce weevil is an extremely difficult pest to manage effectively. Over the years, various control strategies and treatments have been tried, including pesticide spraying, leader clipping, shading, and the use of biocontrols (Nichols 1968, Sundaram 1973, Alfaro *et al.* 1985, Cozens 1987, McLean 1989, Hall 1994). None of these treatments, however, has met with any practical long term success.

Integrated pest management, or IPM, is a strategy that focuses heavily on the interaction between the pest and the host (Retnakaran *et al.* 1982), and attempts to reduce damage to acceptable levels rather than eliminate the pest (Alfaro *et al.* 1995). The employment of this strategy makes use of all available control methods for a particular pest and emphasizes the most environmentally compatible methods. The IPM program developed for the forest pest *Orgyia pseudotsugata* McD. the Douglas-fir tussock moth, is an example of a successful integrated pest management program (Shepherd and Otvos 1986). The use of biological controls, resistant trees, and destruction of overwintering sites were all components of one of the earliest proposed IPM programs for the spruce weevil (Dixon and Houseweart 1982). More recently, Alfaro *et al.* (1995) proposed an in-depth IPM program for *P. strobi* in British Columbia. This system involves a decision support system to evaluate the costs and benefits of different control strategies so that the proper combination of control methods can be utilized depending on the given situation.

1.4 TREE DEFENSES

Throughout history, herbivores have adapted to exploit available nutrient sources. This in turn has resulted in the evolution of defensive traits in the plants under attack, and subsequent offensive counter-adaptations in the consumers (Ehrlich and Raven 1964, Berryman 1988, Rausher 1996).

Berryman (1988) developed an evolutionary theory of plant defense based on "optimal defense theory" which is founded on two assumptions: 1. Defensive adaptations evolve to maximize the inclusive fitness of the individual organism; 2. All defensive traits have a cost in terms of individual fitness because energy or nutrients are diverted away from essential functions such as reproduction, into the manufacture of defensive chemicals or structures. Berryman (1988) therefore infers that a particular characteristic will evolve only if its cost is outweighed by its benefit to the organism's overall fitness. Additionally, the benefit of a given characteristic is dependent upon the impact a given herbivore has on plant fitness in a particular environment.

Rausher (1996) defines three types of costs which are incurred by the development of defensive strategies. The first cost is called "allocation cost," and is the result of plant resources being transferred from growth and reproductive processes to defensive processes. The second cost is an "ecological cost," which occurs when a plant becomes resistant to one herbivore, thereby becoming susceptible to a different herbivore. Rausher (1996) states, however, that these trade-offs in resistance are relatively uncommon. The third type of cost is a trade-off between resistance and tolerance, where resistance is

the average amount of damage experienced by a genotype for a given abundance of natural enemies, and tolerance is the average amount of by which the fitness of a genotype is reduced for a given amount of damage (Rausher 1996).

There are three main ways in which plants can deal with herbivore attack:

1. Tolerate the attack, 2. Avoid the attack, or 3. Defend against the attack.

Tolerance is a passive strategy in which the impact of herbivory is minimal, the cost of an adaptive trait is very high, or the effectiveness of a trait is low; and thus, selection pressure for plant adaptation is zero (Berryman 1988). The tolerance strategy is usually employed where insects feed on non-essential plant tissues, or when senescent tissues are being fed on just prior to their natural death.

The avoidance strategy involves a reduction in the likelihood of damage by avoiding attack some of the time, either through escape in space, escape in time, or through chemical camouflage (Berryman 1988). Escape in space can occur through seed dispersal and is frequently a strategy employed by short-lived plants, while escape in time is often employed by long-lived species and can occur through variable host phenology, such as varying the time of bud flush. Chemical camouflage involves the production of chemicals which mask the plant against or confuse herbivores.

The defense strategy is also commonly employed by plants, and can be either constitutive or induced. Constitutive defenses are akin to fortification, and arise when plants routinely incorporate structures or chemicals into their tissues

for the purpose of deterring, repelling, intoxicating, or interfering with herbivore physiology (Berryman 1988). Plants contain a vast array of chemicals, many of which were formerly believed to be by-products of cellular metabolism, and were thus termed "secondary metabolites;" however, many of these chemicals are now believed to be primary defensive compounds (Berryman 1988). Constitutive defenses are established during times when energy and nutrients are abundant, and can be maintained during times when energy is limited, thus providing a constant defense. As a result, constitutive defenses are expected to arise in longer-lived species, where rapid growth and reproduction is not essential. The synthesis of defensive chemicals, however, has a high cost in terms of plant fitness because they are metabolically expensive, and as a result the cost of constitutive defenses is fixed. Furthermore, Berryman (1988) suggests that although constitutive defenses can be effective against a range of generalist herbivores, they can also exert selective pressure for the evolution of specialist herbivores.

Induced defenses are produced in response to herbivory, and include the removal of nutrients essential to attacking insects, or the production of physical or chemical structures to defend against those insects (Berryman 1988).

Physically defensive structures can include the production of heavily lignified cells which are harder to digest, the production of wound periderms which isolate infections, or the production of traumatic resin ducts (Berryman 1988).

Induction of defenses can result in controlled chemical biosynthesis, in which small quantities of defensive compounds are produced by living cells in

response to injury; or in uncontrolled chemical biosynthesis, in which very large amounts of chemicals are produced by living cells near the injury site, and frequently are released into intercellular spaces and conductive tissues when secretory cells rupture in response to the invader (Berryman 1988). The advantage of induced defenses is that the response intensity can be varied depending on the intensity and persistence of the herbivore attack; however, in contrast to constitutive defenses, the success of the defense depends on current energy availability. Berryman (1988) states that constitutive defenses provide flexibility in when energy is utilized, while induced defenses provide flexibility in how energy is utilized.

There are several ways in which insects adapt to counter plant toxins, one of which is through behavioural adaptation. For example, many insects feed around resin-secreting structures in order to deactivate them or to stop the flow of defensive compounds in the area in which they are feeding (Brattsten 1991, Farrell *et al.* 1991). Other methods insects use to counter plant defenses involve physiological processes such as increased rate of food passage through the body, or by storing plant compounds in special tissues or structures (Brattsten 1991). Additionally, insects sometimes adapt to detoxify ingested plant chemicals. Some insects will inoculate their host with a pathogen in order to weaken the host, and this strategy is often coupled with a mass-attack strategy used to overwhelm the host's defenses (Farrell *et al.* 1991).

Maximum plant fitness is selected for through evolution, and the development of new defensive adaptations will be favoured as long as the cost

of the defensive trait is outweighed by the cost of herbivores on the plant in its current state (Berryman 1988). Plants that have to defend themselves against a number of different organisms generally use compounds that deter or kill by several minor modes of action rather than by one major action, and thus keep the selection pressure as variable as possible (Brattsten 1991). By doing this, plants attempt to maintain stability in resistance over time.

1.5 RESISTANCE OF SPRUCE TO WEEVIL ATTACK

Since the 1930s there has been evidence of resistance to weevil attack in certain spruce genotypes (reviewed by Alfaro 1982, and Alfaro *et al.* 1995). More recent experiments done by the B.C. Ministry of Forests and the Canadian Forest Service have demonstrated host resistance in both Sitka spruce (Alfaro and Ying 1990, Ying 1991) and white spruce (Kiss and Yanchuk 1991, Kiss *et al.* 1994, King *et al.* 1997). Host resistance promises to be a valuable component of Integrated Pest Management System control strategies against the spruce weevil (Alfaro *et al.* 1995).

In the latter half of the 1970s, an extensive spruce survey was undertaken to identify tree genotypes with good growth characteristics. With seed taken from these parent trees, several provenance and progeny trials were established, which later became infested by weevils. Analysis of these trials resulted in the identification of resistant populations and individuals within populations of Sitka spruce. For example, some Sitka spruce provenances from high weevil hazard zones and from the Sitka spruce-White spruce hybridization

zone generally had a high level of resistance to weevil attack. Two of these provenances, Haney and Big Qualicum, showed very high levels of resistance to weevil attack, and were also fast growing (Ying 1991). On the other hand, provenances from the Queen Charlotte Islands, which are not known to have been exposed to *P.strobi*, tended to be highly susceptible to the weevil (Ying 1991). Further evaluation of the Jordan River and Cowichan Lake progeny trials suggest that the expression of resistance is strongest in a concentrated area near to the initial Big Qualicum seed collection area (King *et al. Submitted*). Although resistance can be affected by environmental factors, it is known to be strongly genetically based (Kiss and Yanchuk 1991, Ying 1991, Alfaro *et al.* 1993, King *et al.* 1997).

To successfully make use of resistance in Integrated Pest Management, we must understand the mechanisms by which these trees influence weevil behaviour. Several host characteristics that have implications for resistance to *P. strobi* have been summarized by Alfaro (1994). These characteristics include the visual and chemical stimuli involved in the host selection process (Anderson and Fisher 1956, 1960, VanderSar and Borden 1977a, 1977b, 1977c), feeding stimulants and repellents (Alfaro *et al.* 1980, Alfaro 1996a, Tomlin and Borden 1996), oviposition stimulants and repellents (VanderSar 1978, Tomlin and Borden 1996), reproductive inhibitors (Sahota *et al.* 1994, 1998), resin properties (Santamour and Zinkel 1977), host and insect phenology (Hulme 1995, Alfaro *et al.* 1999), and leader morphology, such as length, diameter, bark thickness, and needle density (Plank and Gerhold 1965, Silver 1968, Harris *et*

al. 1990, Alfaro *et al.* 1993, Tomlin and Borden 1997a). In addition to the external morphology of the leader, internal leader characteristics, such as the size and density of cortical resin canals and bark thickness have been associated with resistance (Stroh and Gerhold 1965, Tomlin and Borden 1994, Alfaro 1996a, Alfaro *et al.* 1997, Tomlin and Borden 1997a). Cortical resin ducts are involved in the production of constitutive resin which is used to repel and drown invading insects; but trees are also capable of producing a traumatic reaction to supplement these constitutive defenses (Berryman 1972, 1988, Alfaro 1995, Alfaro *et al.* 1996b, Tomlin *et al.* 1998).

It has been widely reported that a tree's defenses can vary depending on the time of year (Cerezke 1972, Cates and Alexander 1982, Stephen and Paine 1985, Langstrom *et al.* 1992, Hulme 1995, Alfaro 1996a, Alfaro *et al.* 1996b, Alfaro *et al.* 1997), and there are indications that, in some genotypes, resistance may be transient rather than absolute (Hulme 1995, Alfaro 1996a). Thus, the overall objective of this study is to determine how the constitutive and induced resin defenses in Sitka spruce vary through the growing season, and to determine how these defenses differ between resistant and susceptible trees.

2. PATTERNS OF GROWTH AND PHENOLOGICAL DEVELOPMENT IN THE LEADERS OF RESISTANT AND SUSCEPTIBLE SITKA SPRUCE FAMILIES

2.1 INTRODUCTION

Growth and phenology are known to be under genetic control in many conifer species, and these characteristics can vary among different provenances or seed sources. For example, certain provenances of Sitka spruce have been found to have greater rates of growth than others (Genys 1965, Burley 1966a, Teich *et al.* 1975, Pollard and Ying 1979, Cahalan 1981), and this variation in growth has been found to be highly correlated with the latitude of the seed source (Burley 1966b, Ying 1991). Similar findings regarding growth have been made for other species of spruce as well (Johnsen and Seiler 1996, Stoehr *et al.* 1998). Latitude, elevation, and other environmental factors of the seed source location have also been found to affect budbreak in Sitka spruce and other species in common garden experiments (Burley 1966a, Burley 1966b, Nienstaedt and King 1970, Perry 1971, Cannell and Willett 1975, Pollard and Ying 1979, White *et al.* 1979, Worrall 1983, Kiss and Yanchuk 1991, Dunlap *et al.* 1992, Murray *et al.* 1994, Johnsen and Seiler 1996, King *et al.* 1997).

A study of interior spruce families of known resistance or susceptibility to *Pissodes strobi*, revealed that the resistance ranking of a family was significantly correlated with the elevation and latitude of the parent trees (Alfaro *et al.* 1996a, King *et al.* 1997). This indicates that there could be a strong relationship between weevil resistance and tree growth or phenology (Alfaro *et al.* 1996a).

It is frequently stated that weevils prefer longer, thicker leaders, and this has been observed for both spruce and pine hosts (Silver 1968, VanderSar and Borden 1977c, Wilkinson 1983, Wallace and Sullivan 1985, Alfaro 1989b, Alfaro and Ying 1990, Alfaro *et al.* 1993, van den Driessche 1997). Kiss and Yanchuk (1991), however, found that faster growing families of interior spruce are less frequently damaged than slow growing families. A closer look at this apparently contradictory evidence revealed that while there is a significant positive phenotypic correlation between leader growth and weevil attack, there is a strong negative genetic correlation between leader growth and weevil attack (King *et al.* 1997). King *et al.* (1997) suggest that the positive phenotypic correlation between weevil attack and leader size is related to the weevils selecting the larger leaders to ensure that the maximum amount of food is available for the larvae, and therefore offspring production and survival should be optimum. The negative genetic correlation between weevil attack and leader growth is a function of more vigorous families being able to produce more effective defensive responses (King *et al.* 1997). The *apparency theory* of plant defense suggests that species that are easily found by herbivores must invest in defense to a greater degree than those species which are less easily found (Feeny 1976). This may also be true on an intraspecific level. Through coevolution, the weevils preference for longer leaders could result in faster growing families of spruce developing better resistance mechanisms.

Tree phenology is an important factor determining the success of insect attacks at different times during the year, and it can have a dramatic effect on

food availability for developing insects. In pedunculate oak, trees that break bud earlier support greater spring populations of *Operophtera brumata* L. and *Tortrix viridana* L. caterpillars and sustain greater defoliation than those with delayed budbreak (Hunter 1992). This can be related to the fact that earlier flushing trees made food available to the hatching caterpillars sooner than the late flushing trees, and thus allowed the insect population to build to a greater level on these trees.

The impact of host plant phenology on larval survival is also well known for other insect species (Shepherd 1985, Watt 1987, Hunter 1990, Hunter 1992). Not only does phenology affect the quality and quantity of food available at specific times of year, but it also affects the production of resin, terpenes, and other defensive or attractive compounds (Gara *et al.* 1971, Kramer and Kozlowski 1979, Walter *et al.* 1989, Hulme 1995, Tomlin 1996, Alfaro *et al.* 1999). For example, initiation of cambial activity and bud elongation have been found to be necessary for active secretion in xylem resin canals or production of traumatic resin canals (Safranyik *et al.* 1983, Walter *et al.* 1989). Similarly, Tomlin (1996) discovered that attraction or repulsion of *P. strobi* is lost when buds, which contain several highly volatile monoterpenes, are removed from Sitka spruce twigs.

A Sitka spruce provenance trial at Fair Harbour, British Columbia revealed that the resistant provenances broke bud earlier than susceptible provenances, and that earlier flushing trees tended to be less damaged by the weevil (Hulme 1995). Similar findings were made by Alfaro *et al.* (1999) for a

different set of resistant families. Hulme (1995) observed that resistant trees were able to produce more resin at the time that weevils normally oviposit, indicating that early flushing could allow for earlier development of defensive mechanisms. This was supported by the fact that leaders from resistant clones were damaged when weevils were caged on the leaders in early spring before resin flow became high, and leaders of susceptible clones frequently avoided damage when oviposition was delayed (Hulme 1995). Sullivan (1960) found, in eastern white pine (*Pinus strobus* L.), that low temperatures can slow the development of weevil larvae, allowing them to be easily drowned by resin and causing significant brood mortality. Younger larvae seem to be more susceptible to resin drowning than older larvae. Overhulser and Gara (1981) suggest that cooler temperatures on coastal Sitka spruce, which causes slower development of weevil broods, would increase the effectiveness of host resistance by increasing brood exposure to host resins. Furthermore, when wounding trees at monthly intervals, Safranyik *et al.* (1983) found low resin flow early in the season and suggested that it would be advantageous for spruce beetles (*Dendroctonus rufipennis* Kirby) to colonize during this time.

In addition to bud flushing and resin production, radial and apical growth of the leader over time is an important factor in resistance of Sitka spruce to the white pine weevil. For example, the resistance of a leader, with respect to resin canal density, has been found to decrease as the leader ages one or more years (Alfaro 1996a). Alfaro (1996a) observed that weevils will preferentially chose to feed and oviposit in the leaders of susceptible trees rather than leaders of

resistant trees, however, when restricted to resistant trees, the weevils will feed and oviposit in the previous year's internode. Additionally, it was found that there was a higher number per unit area of bark and a greater proportional area of resin canals in a given year's leader compared to the previous year's internode (Alfaro 1996a). This leads to the assumption that there is a dilution of (or stretching of the spaces between) cortical resin canals as the tree undergoes radial growth. Blanche *et al.* (1992) observed a similar phenomenon in loblolly pine, *Pinus taeda* L., with the horizontal and vertical xylem resin duct densities being negatively correlated with radial growth increment.

Burley (1966b) suggests that the main characteristics affecting apical growth are intrinsic rate of growth, the length of the growing period, and the environmental conditions during the growing season. Different provenances of Sitka spruce have been observed to show different rates and patterns of seasonal growth (Burley 1966a, b). If this variation in growth were present among different families of Sitka spruce, it could have significant implications for resistance to the spruce weevil. For example, trees with rapid radial growth early in the season, could undergo a more rapid dilution of constitutive resin canals than trees that have slower, or delayed secondary growth. It is possible then that resistant trees have inherently slower radial growth than susceptible trees early in the season and are therefore better able to defend against weevil attack during the peak oviposition period.

Trees that flush earlier are not necessarily faster growing trees.

Nienstaedt and King (1970) found a negative correlation between flushing time

and the length of the growth period in white spruce. Aitken and Hannerz (*In press*) suggest that growing season length is more closely related to the cessation of growth than to the initiation of growth in most conifers; and Cahalan (1981) found no correlation between date of flushing and bud set in Sitka spruce. The relationship between bud phenology of Sitka spruce and resistance to the white pine weevil has been examined by Hulme (1995) and Alfaro *et al.* (1999), but the relationship between bud phenology and radial growth rate of the shoot as a function of resistance to the white pine weevil has not yet been explored.

The objectives of this study are: 1. To test the hypothesis that resistant families of Sitka spruce will have delayed or slower radial and apical growth early in the season when compared to susceptible families. 2. To examine the relationship between growth and bud phenology in Sitka spruce and determine how they are related to resistance to *P. strobi*.

2.2 MATERIAL AND METHODS

2.2.1 Study Sites and Genetic Material

This study was undertaken from March to August 1998 at Sitka spruce progeny trials established in the winter of 1991-92 at Jordan River (48° 25' N, 124° 01' W; 120 m elevation; 5° slope) and Meades Creek, on the North arm of Cowichan Lake, (48° 51' N, 124° 07' W; 190 m elevation; flat terrain) on Vancouver Island, British Columbia, Canada. Both sites are within the Very Dry

Maritime subzone of the Coastal Western Hemlock biogeoclimatic zone (Nuszdorfer and Boettger, 1994).

Both plantations contain the same 75 open-pollinated families of Sitka spruce grown from open-pollinated seeds collected from locations ranging from the Queen Charlotte Islands and coastal BC to Oregon, USA. Each family was replicated in 24 blocks at each site. A report by Alfaro *et al.* (1998) ranked individual families as resistant, susceptible, or intermediately susceptible to *P. strobi*. This ranking is an attribute of the family as a whole, and not of individual trees within the family. Four resistant families (No.'s 12, 17, 24, 120) and four susceptible/intermediately susceptible families (No.'s 3, 11, 41, 99) were selected for this study, and all families except family 41 are open pollinated. Family 41 is from a seedlot, collected from a minimum of 20 trees within a range of 10 miles and 300 m elevation. All families used in this study belong to the Big Qualicum Provenance except family 41 which is from the West Vancouver Island provenance. The four resistant families were chosen because, at both Jordan River and Cowichan Lake, less than 25% of the trees in each family had been successfully attacked by weevils. The susceptible families were randomly selected from the remaining pool of families where greater than 25% of the trees in each family had been successfully attacked by weevils. Resistant families had been identified as those with less than 25% of the trees successfully attacked in a previous study which was done for the BC Ministry of Forests using the same families (Alfaro *et al.* 1998).

2.2.2 Data Collection

Periodic measurements of 1997 (previous year's) leader diameter growth were taken at the midpoint of the leader using Vernier callipers. At the same time, the developmental stage of the apical bud was recorded for each tree using the eight-stage classification system developed by Alfaro *et al.* (1999) (Figure 2.1). Measurements were made bi-weekly from March 14 to April 20, 1998, weekly until July 14, and then bi-weekly until August 26. Thus, the frequency of sampling was higher during the period of rapid growth than at the start or at the end of the season. Temperature data was recorded hourly for each plantation using a Stowaway® data logger starting March 1, 1998. Degree-days were calculated from the hourly weather data using a program similar to that developed by Raworth (1994), using a 5° C developmental threshold temperature. Both programs are based on the sine method described by Frazer and Gilbert (1976). Cannell and Smith (1983) used the same 5° C threshold in their prediction of budburst phenology of Sitka spruce in Britain. The presence of weevil activity and quantity of weevil feeding and oviposition on leaders was noted, in an effort to determine the period of peak oviposition.

2.2.3 Data Analyses

To determine the effect of weevil resistance on leader length, an analysis of variance (ANOVA) was performed using the STATISTICA® program from StatSoft, Inc. (2300 East 14th St., Tulsa, OK 74104). The mean diameter of the 1997 internode at the end of the study was compared for resistant and

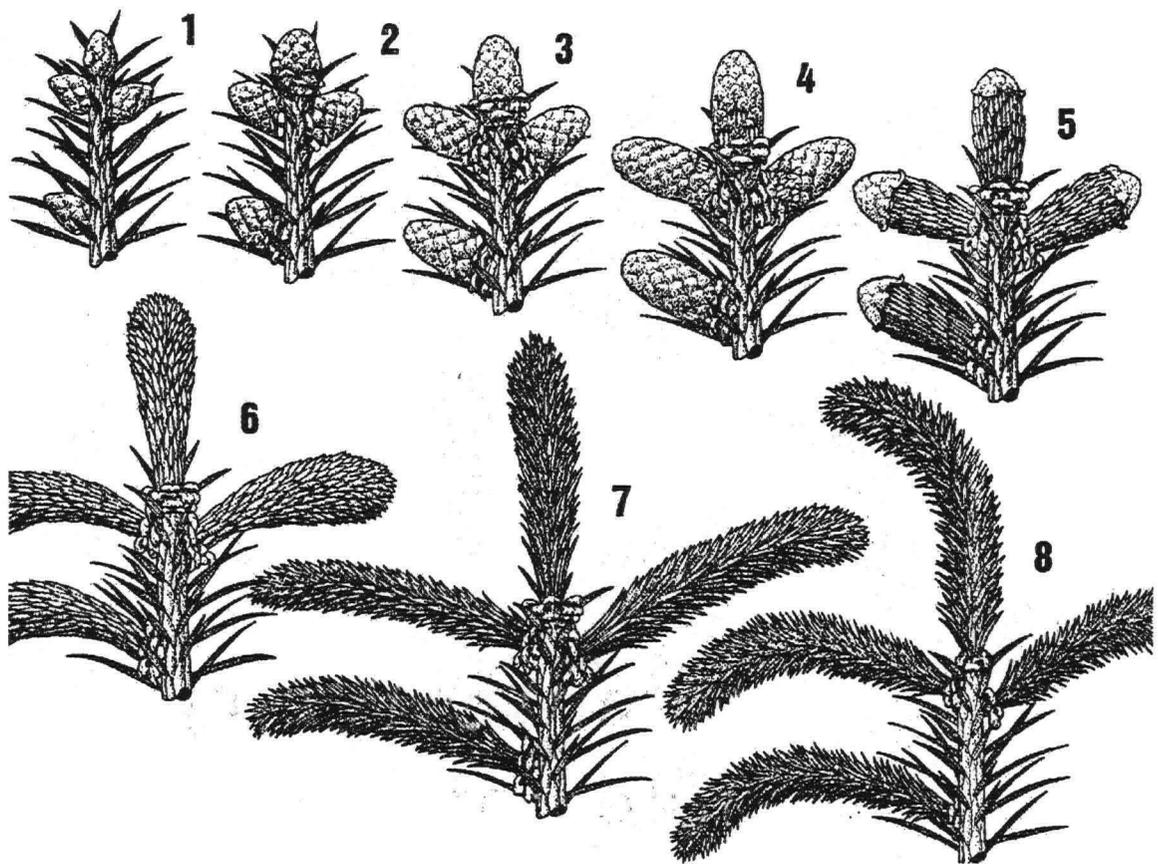


Figure 2.1. Budburst classification guide depicting phenology stages 1 through 8 (adapted from (Shepherd 1983)). Budburst phenology stages described as follows: (1) *shiny conical* – buds slightly conical with scales starting to peel back; (2) *shiny/swollen* – buds similar to (1) but more swollen; (3) *yellow/swollen* – buds considerably swollen and appear yellowish in colour; (4) *columnar* – shoots starting to elongate, bud scales transparent so that the green colour of the needles is clearly visible; (5) *split* – shoot elongating, bud cap substantially splitting but still attached to tip of shoot; (6) *brush* – bud cap usually no longer present, needles appear to originate from one point; (7) *feather* – needle bases separate; and (8) *growing shoot* – needles widely separated out from expanding shoot. (Copied with permission from Alfaro *et al.* 1999.)

susceptible families using an analysis of covariance (ANCOVA). The covariate used in this case was the diameter of the internode at the beginning of the season. A repeated measurements ANOVA was used to determine if radial growth of the leader over the course of the study was related to resistance. The data was then broken into three sections: the period prior to peak weevil oviposition, the period during peak oviposition, and the period following peak oviposition. Using the repeated measures ANOVA, these three sections of the data were examined separately to determine if radial growth was significantly different among resistant and susceptible families during any of these time periods. The model used in the repeated measures ANOVA was:

$Y_{ijk} = \mu + R_i + S_j + RS_{ij} + \epsilon_{(ij)k}$ where Y_{ijk} represents the variable being measured (i.e. radial growth); μ was the population mean; R_i was the resistance status of the tree (a fixed variable), S_j was the sampling date (a fixed variable), RS_{ij} was the interaction between the resistance status of the tree and the sampling date, and $\epsilon_{(ij)k}$ was the experimental error. The same procedure and model were used to determine if rate of bud flush could be related to resistance or susceptibility.

Finally, to determine if there was any correlation between flushing rate and growth rate, Pearson product-moment correlations were estimated among traits for trees from resistant families, trees from susceptible families, and for all trees combined.

2.3 RESULTS

Observations made in the field regarding weevil mating and the presence of eggs on leaders indicated that the peak weevil oviposition period occurred from approximately April 30 to June 22, between approximately 210 and 470 degree days from March 1st using the threshold temperature of 5° C.

No significant difference was found between trees from resistant and susceptible families in the final length of the 1997 leader ($F_{(1,190)}=0.41$, $p=0.52$). This finding was held true when individual families were compared as well ($F_{(7,184)}=1.09$, $p=0.37$).

Similarly, a comparison of the 1997 internode diameter at the end of the summer revealed no significant difference between the trees from resistant and susceptible families, even when leader diameter at the beginning of the season was used as a covariate ($F_{(1,177)}=1.85$, $p=0.18$).

Examination of diameter growth of the 1997 internode over the course of the season did not show any significant differences between trees from resistant and susceptible families ($F_{(1,178)}=0.0$, $p=0.85$) (Table 2.1)(Figure 2.2), nor did it show any differences among the families ($F_{(7,172)}=0.60$, $p=0.76$). These findings also held true when the two sites were examined separately (Jordan River: $F_{(1,88)}=0.13$, $p=0.72$; Cowichan Lake: $F_{(1,88)}=0.01$, $p=0.91$) (Figure 2.3). When diameter growth was examined for the time prior to peak weevil oviposition ($F_{(1,186)}=0.29$, $p=0.59$), the time during peak oviposition ($F_{(1,186)}=0.07$, $p=.79$), and the time after peak oviposition ($F_{(1,178)}=0.23$, $p=0.63$), no difference was found between trees from resistant and susceptible families.

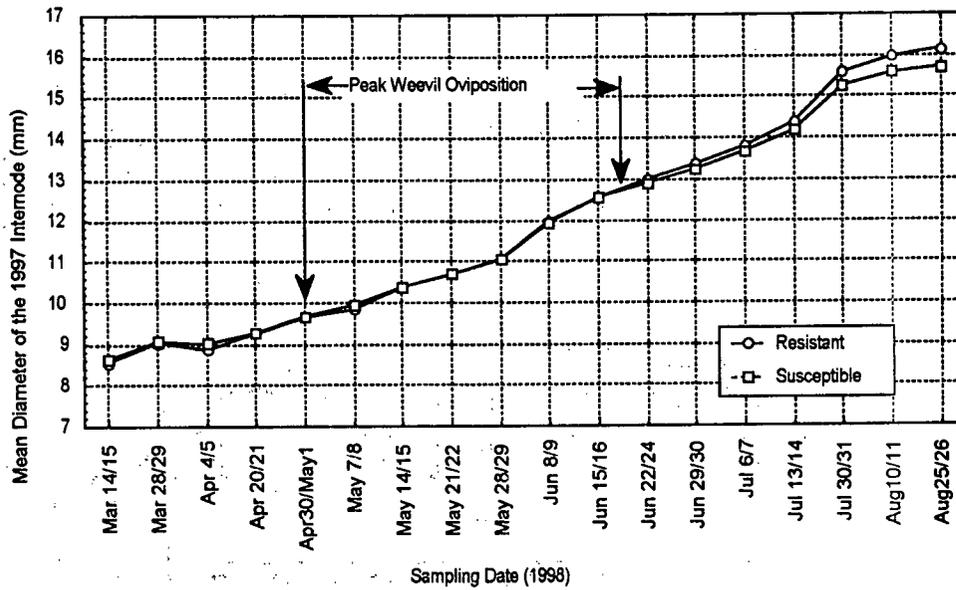


Figure 2.2. Mean diameter growth of the 1997 internode for resistant and susceptible trees pooled for both sites.

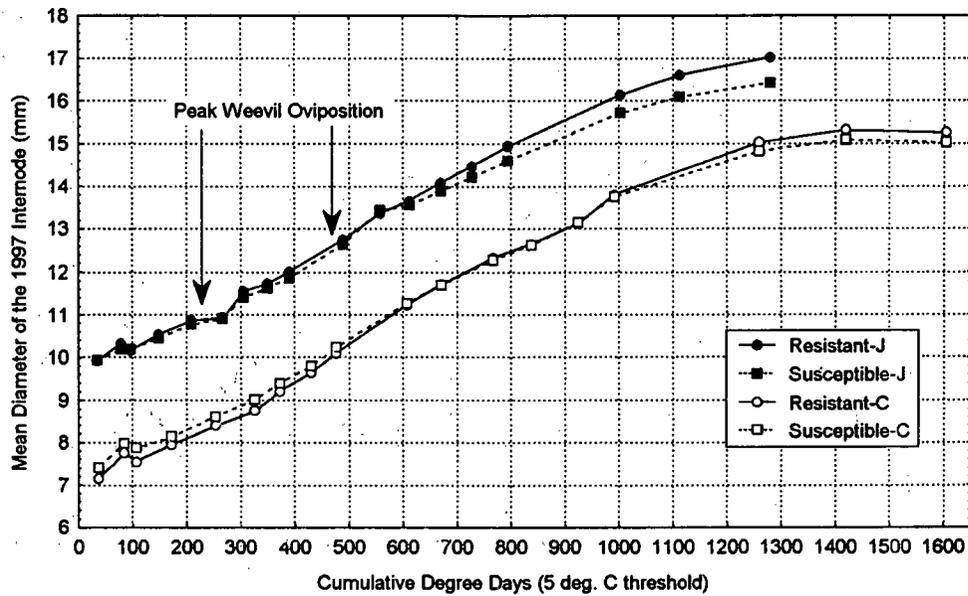


Figure 2.3. Mean diameter growth of the 1997 internode for resistant and susceptible trees at both Jordan River and Cowichan Lake.

Table 2.1. Mean values (\pm Standard Error of the Mean) for the 1997 internode diameter (mm) of resistant and susceptible families of Sitka spruce at eighteen different times during the growing season (T1-T18).
 R=resistant (n=95), S=susceptible (n=85).

	Mar 14/15	Mar 28/29	Apr 4/5	Apr 20/21	Apr 30/May 1	May 7/8	May 14/15	May 21/22	May 28/29
R	8.55 \pm 0.24	9.05 \pm 0.23	8.87 \pm 0.23	9.25 \pm 0.23	9.64 \pm 0.23	9.85 \pm 0.23	10.39 \pm 0.24	10.70 \pm 0.24	11.07 \pm 0.24
S	8.66 \pm 0.25	9.07 \pm 0.24	9.02 \pm 0.25	9.28 \pm 0.24	9.67 \pm 0.25	9.96 \pm 0.25	10.39 \pm 0.26	10.70 \pm 0.27	11.05 \pm 0.28

	Jun 8/9	Jun 15/16	Jun 22/24	Jun 29/30	Jul 6/7	Jul 13/14	Jul 31/31	Aug 10/11	Aug 25/26
R	11.99 \pm 0.26	12.55 \pm 0.27	13.00 \pm 0.28	13.37 \pm 0.30	13.81 \pm 0.30	14.38 \pm 0.31	15.59 \pm 0.35	15.96 \pm 0.36	16.15 \pm 0.38
S	11.95 \pm 0.30	12.56 \pm 0.33	12.91 \pm 0.34	13.25 \pm 0.35	13.68 \pm 0.36	14.18 \pm 0.37	15.27 \pm 0.40	15.58 \pm 0.42	15.71 \pm 0.44

Bud phenology data did not follow a normal distribution; however, when an arcsine transformation was done, there was no significant change in the normality of the data. According to Zar (1996): "The analysis of variance is robust with respect to the assumption of the underlying populations' normality. The validity of the analysis is affected only slightly by even considerable deviations from normality, especially as n increases."

A statistically significant relationship ($F_{(17,2822)}=1.8$, $p=0.02$) was observed between apical bud development and resistance. A plot of this relationship illustrated that during the periods prior to ($F_{(1,190)}=0.39$, $p=0.53$) and following peak weevil oviposition ($F_{(1,167)}=3.03$, $p=0.08$) there was little difference between the resistant and susceptible trees, but that during the peak oviposition period a significant difference was present (Figure 2.4). When trees from resistant and susceptible families were compared for the peak weevil oviposition period it was found that the apical buds of the resistant trees had a higher mean developmental stage ($F_{(1,184)}=3.8$, $p=0.05$) (Table 2.2).

Correlation analysis revealed that there was no overall correlation between bud flush and radial growth of the leader. This was true for resistant trees, susceptible trees, and all trees combined (Table 2.3). A significant negative correlation was observed at the third measurement date in all categories, and a positive correlation was found at the ninth measurement date in susceptible trees. It is possible that the few individual correlations observed could be chance correlations.

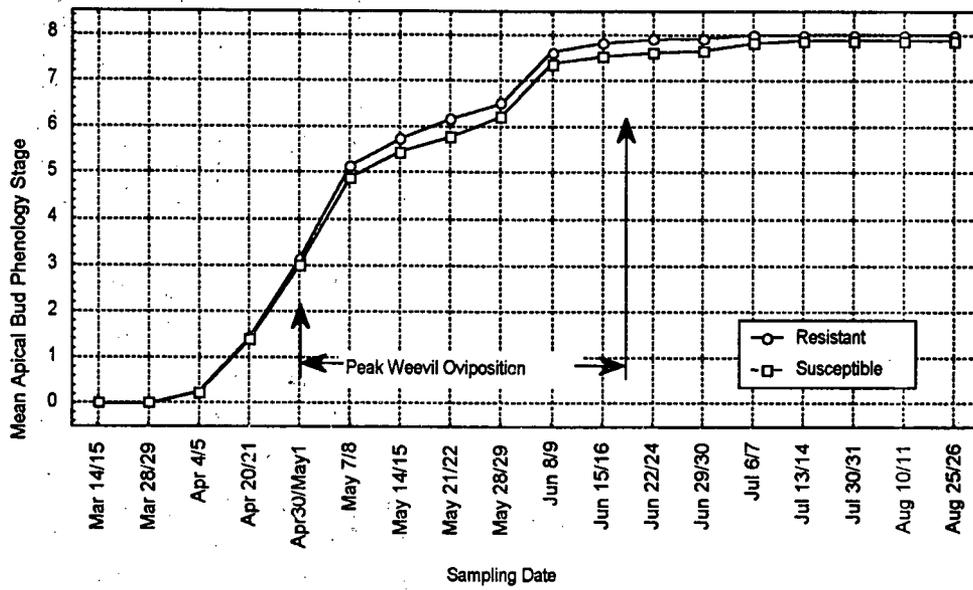


Figure 2.4. Mean developmental stage of the 1998 apical leader bud for resistant and susceptible trees pooled for both sites.

Table 2.2. Mean values (\pm Standard Error of the Mean) for the developmental stage of the 1998 apical bud of resistant and susceptible families of Sitka spruce at eighteen different times during the growing season (T1-T18). R=resistant (n=87), S=susceptible (n=81). * Indicates significance at $\alpha=0.10$; ** Indicates significance at $\alpha=0.05$.

	Mar 14/15	Mar 28/29	Apr 4/5	Apr 20/21	Apr 30/May 1	May 7/8*	May 14/15*	May 21/22**	May 28/29*
R	0.00 \pm 0.00	0.00 \pm 0.00	0.25 \pm 0.05	1.43 \pm 0.08	3.10 \pm 0.14	5.15 \pm 0.11	5.74 \pm 0.07	6.17 \pm 0.06	6.51 \pm 0.08
S	0.00 \pm 0.00	0.00 \pm 0.00	0.25 \pm 0.05	1.38 \pm 0.09	2.99 \pm 0.15	4.89 \pm 0.14	5.46 \pm 0.12	5.78 \pm 0.12	6.22 \pm 0.12

	Jun 8/9*	Jun 15/16**	Jun 22/24**	Jun 29/30**	Jul 6/7*	Jul 13/14	Jul 31/31	Aug 10/11	Aug 25/26
R	7.61 \pm 0.06	7.80 \pm 0.04	7.89 \pm 0.03	7.92 \pm 0.03	7.98 \pm 0.02	8.00 \pm 0.00	8.00 \pm 0.00	8.00 \pm 0.00	8.00 \pm 0.00
S	7.36 \pm 0.13	7.51 \pm 0.13	7.59 \pm 0.13	7.64 \pm 0.13	7.80 \pm 0.10	7.88 \pm 0.10	7.88 \pm 0.10	7.88 \pm 0.10	7.88 \pm 0.10

Table 2.3. Correlations between leader apical bud development and radial growth for resistant trees, susceptible trees, and all trees combined, at 18 times during the growing season. Correlation coefficients significant at the 0.05 level of probability are indicated by *. No correlations were possible where correlation coefficients are represented by "--".

	Mar 14/15	Mar 28/29	Apr 4/5	Apr 20/21	Apr 30/May 1	May 7/8	May 14/15	May 21/22	May 28/29
R&S	--	--	-0.27 *	-0.06	-0.04	0.03	-0.02	0.04	0.14
R	--	--	-0.29 *	-0.09	-0.13	-0.03	-0.05	0.01	0.02
S	--	--	-0.25 *	-0.04	0.06	0.09	0.00	0.05	0.24 *

	Jun 8/9	Jun 15/16	Jun 22/24	Jun 29/30	Jul 6/7	Jul 13/14	Jul 31/31	Aug 10/11	Aug 25/26
R&S	0.11	0.07	0.09	0.10	0.03	0.05	0.06	0.07	0.08
R	0.09	-0.05	0.10	0.00	-0.13	--	--	--	--
S	0.12	0.12	0.09	0.13	0.06	0.07	0.08	0.09	0.11

2.4 DISCUSSION

The lack of any significant difference in leader length between trees from resistant and susceptible families differs from previous findings regarding weevil leader length preference. For interior spruce, King *et al.* (1997) determined that leader length is correlated with weevil resistance, however, evidence found here indicates that this trait can not consistently be used as an indicator of resistance or susceptibility to weevil attack in Sitka spruce. Instead, leader length is likely an indicator of the overall resistance status of the tree only for some genotypes or for other spruce species.

Through laboratory bioassays and measurement of dormant trees in the field, Sullivan (1961) determined that weevils prefer thicker leaders irrespective of length in eastern white pine. The current study, however, did not reveal any difference between resistant and susceptible families in the end of season leader diameter. The significance of these findings must be considered carefully because measurements made at only a single time during the year, which are often used as a measure of tree vigour or growth, do not accurately portray patterns of growth and development in the field. This is especially important when dealing with insect resistance traits, as measurements of tree characteristics must coincide with key periods of insect activity (e.g. oviposition, larval feeding) to be of value.

As a tree undergoes radial growth, its leader increases in diameter, but no new cortical resin canals are produced, and thus there are fewer resin canals per unit area of bark as the tree grows. This dilution of constitutive resin canals

through growth is known to affect a tree's susceptibility to weevil attack Alfaro (1996a). Stroh and Gerhold (1965) observed that weevils avoided severing resin duct epithelial cells when making feeding punctures, and Alfaro (1996a) found that the density of constitutive resin canals decreases as a leader ages one or more years. In this study, resistant and susceptible families of Sitka spruce demonstrated no significant difference in growth rate at any time during the season. Thus, retarding the dilution of cortical resin ducts through delayed or slow early season radial growth is not likely to be a mechanism of resistance to weevil attack in these families. The effects of seasonal growth on the structure of the cortical resin system will be covered in the Chapter 3.

The findings that resistant trees have slightly more rapid apical bud flushing than susceptible trees agree with the findings of Hulme (1995) and Alfaro *et al.* (1999). Apical bud phenology is likely an indicator of the timing of other physiological processes in the plant. Because hormones (primarily auxin) from active buds are known to be involved in the initiation of cambial activity (Kramer and Kozlowski 1979, Savidge 1988), it is probable that resistant trees are able to activate the production of resin, repellents, and other defensive mechanisms more rapidly than susceptible trees. Further studies must be undertaken in order to evaluate the validity of these hypotheses before the correlation of tree apical bud phenology with resistance can be fully understood.

The fact that no correlation was found between bud flush and secondary growth of the leader is not unexpected as similar observations have been made for other conifer species (Hanover 1963). While the initiation of cambial growth

may be partly regulated by phenology, the continuation of radial growth is likely governed by environmental factors such as temperature and availability of water and nutrients (Burley 1966a, Kramer and Kozlowski 1979, Lavender 1981, Blanche *et al.* 1992). The lack of a relationship between the two factors appears to be of minor importance in understanding resistance of Sitka spruce to the weevil given the fact that no difference was found in the seasonal radial growth rates of trees between resistant and susceptible families.

3. CONSTITUTIVE DEFENSES, INDUCED DEFENSES, AND GROWTH

3.1 INTRODUCTION

Plant resin is known to be a major factor in the resistance of conifers to insects. Berryman (1972) described two different mechanisms by which resin is used in conifer defense. The first mechanism is a constitutive or primary resin system, which consists of preformed cortical and xylem resin ducts. Severing of these ducts results in passive flow of resin which serves to cleanse and seal wounds, as well as to repel invading insects (Berryman and Ashraf 1970, Christiansen and Horntvedt 1983, Berryman 1988, Baier 1996, Alfaro *et al.* 1997, Tomlin and Borden 1997a, Cornelissen and Fernandes 1998). The secondary mechanism of defense is referred to as an induced, hypersensitive, or traumatic response. The induced defense response involves morphological and physiological changes that result in the premature death or necrosis of tissue infected by a foreign agent, as well as the containment and inactivation of that agent (Wong and Berryman 1977, Paine 1984, Fernandes 1990, Alfaro 1995, Alfaro *et al.* 1996b, Baier 1996, Cornelissen and Fernandes 1998, Tomlin *et al.* 1998).

Callaham (1960) reported that high levels of resin flow repelled beetles from feeding sites in Ponderosa pine and Silver (1968) observed that resin flow was stimulated by feeding and oviposition of *Pissodes strobi*. Resinosis is also believed to be the primary reason for brood mortality for *P. strobi* (Sullivan 1960, Overhulser and Gara 1981, Dixon and Houseweart 1982).

In order to properly understand the nature of resinosis and its potential as a mechanism of resistance to the white pine weevil it is first necessary to understand the organization and function of the resin canal system. Ruel *et al.* (1998) observed that constitutive resin flow in loblolly pine increased following mechanical wounding. In cases of severe wounding, resin flow decreased immediately after wounding; but began to increase and kept increasing following the initial decrease. These observations seem to coincide with the findings of Walter *et al.* (1989), who found that, in pine trees, the secretory activity of the cortical resin ducts (which originate from the apical meristem) is transient and terminates when the shoot has grown a full year. As the ducts age, the secretory features of the epithelial cells are progressively lost, however wounding can induce a new secretory cycle and the recovery of secretory structures (Walter *et al.* 1989). Walter *et al.* (1989) noticed swelling of the epithelial cells and dedifferentiation of secretory structures as early as two days after wounding. The resin flow observed by Ruel *et al.* (1998) in the first day following wounding was probably the flow of resin stored in the ducts, and active secretion of resin was probably not observed until the second day. Wounding does not normally result in the production of new resin ducts or isolated secretory cells in the cortex (Walter *et al.* 1989).

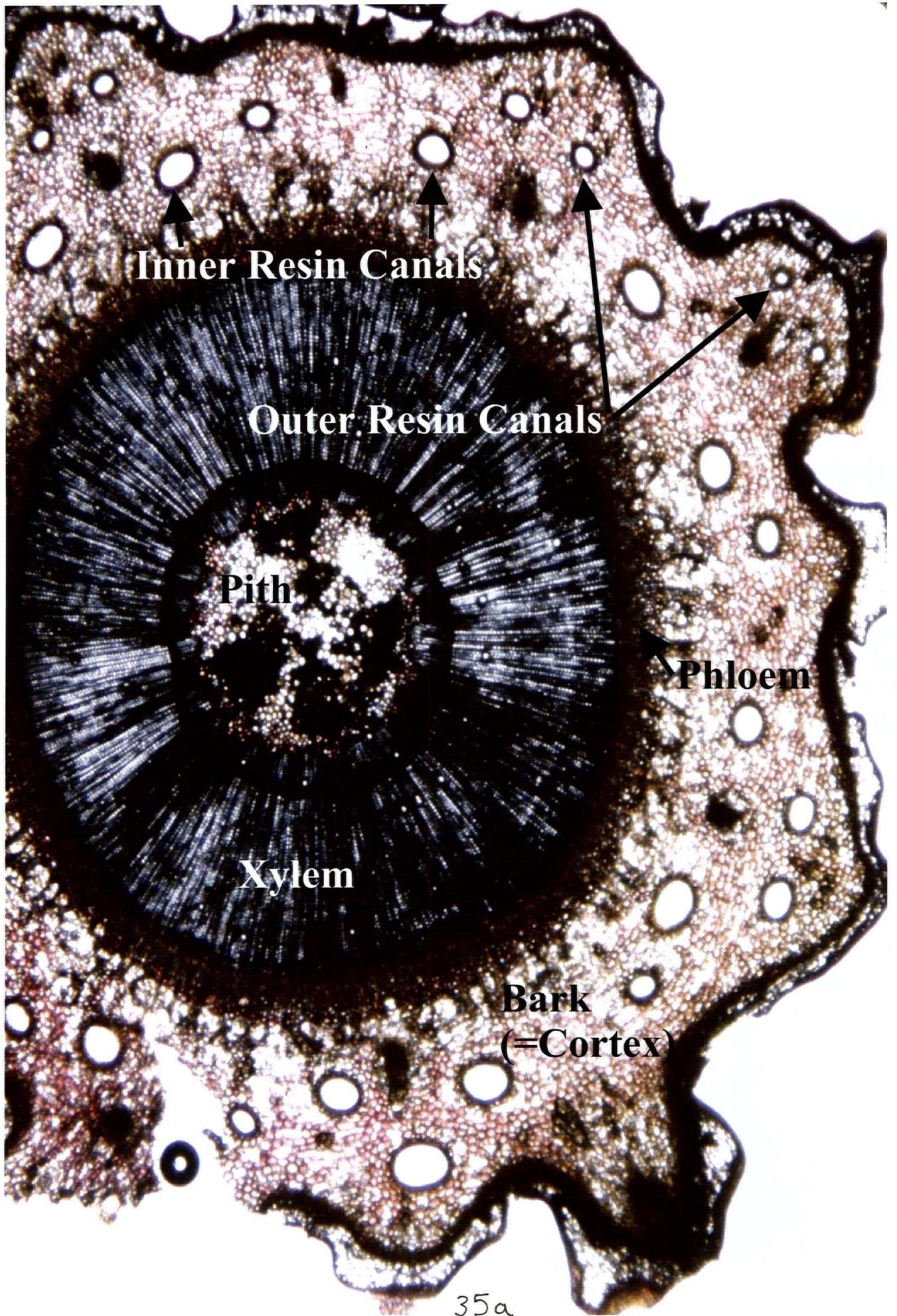
Initial exudation of constitutive resin is thought to depend on the storage capacity of the resin duct system and on oleoresin viscosity, and to a much lesser extent on exudation pressure (Hodges and Lorio 1971, Christiansen *et al.*

1987). The storage capacity of the resin duct system in some conifers has been related to the number and size of cortical resin ducts (Schopmeyer *et al.* 1953).

In *Picea* spp., the resin ducts are distributed in the cortex and secondary xylem of stems and in the mesophyll. They are formed schizogenously, and mature ducts are elongated structures composed of epithelial cells surrounding an intercellular space and surrounded by one to three layers of sheath cells (Figure 3.1). Cortical resin ducts are produced during bud growth, and the epithelial and sheath cells of these ducts are alive and thin-walled. Longitudinal and radial resin ducts in the secondary xylem of stems are produced by cambial activity. The longitudinal ducts are in the outer portion of the earlywood and the first-formed latewood. The sheath cells of longitudinal ducts have thick, lignified walls and some remain active for several years. The epithelial cells of the vertical resin canals are also primarily thick-walled. The radial resin ducts occur in xylem rays and the sheath cells also have thick, lignified walls. The epithelial cells of the radial ducts can be either thin- or thick-walled and lignified (Wu and Hu 1997). Epithelial cells are responsible for the secretion of resin (Werker and Fahn 1969).

Tomlin and Borden (1994) classified two different types of cortical resin canals based on their size and location. Inner resin canals are defined as large, uniformly sized resin ducts observed in a ring closest to the xylem tissue. Outer resin canals are those peripheral to the inner ducts, and are usually smaller and more variable in size.

Figure 3.1.: Cross section of a Sitka spruce lateral showing inner and outer cortical resin canals (40X).



Jou (1971) found that the number of cortical resin canals in Sitka spruce increased from the top to the bottom of a given internode, and that the mean size of resin canals decreased from the top to the bottom of an internode. As a stem ages, it increases in diameter and the tangential distance between the resin canals increases but the size of individual resin canals is thought to remain constant, thus creating a dilution of resin canals with age of the internode (Jou 1971). This dilution effect was also observed by Alfaro (1996a).

An extensive pattern of cortical resin canal branching has been observed in Sitka spruce, as well as some fusion of adjacent resin canals, and in some instances, the spontaneous origin of resin canals near the outer edge of the cortex (Jou 1971). Jou (1971) observed that larger inner resin canals often branched to form two smaller ones, with the larger of the two branches remaining in the inner ring of canals and the smaller of the branches moving outward in the cortex. The smaller one usually underwent a second branching and movement outward to form a pair of outer resin canals.

Studies of leaders attacked by weevils indicated that almost all punctures contacted one or more resin canals (Overhulser 1973), but that the punctures never severed the epithelial cells of the canals (Stroh and Gerhold 1965). Stroh and Gerhold (1965) believed that if a weevil began feeding directly over an outer resin duct then feeding would cease when the epithelial cells of the duct were encountered. If feeding began to either side of the outer duct, then excavation would curve around the duct in order to avoid the epithelial cells. They further believed that if an inner resin duct was encountered during feeding that the

direction of excavation would be reoriented and then feeding would continue. If the inner and outer resin ducts were close together, then feeding would not be able to continue around the inner ducts without contacting the epithelial cells of the outer ducts (Stroh and Gerhold 1965). The frequency of contact with the inner ducts was related to the weevil enlarging the feeding cavity between the inside canals (Stroh and Gerhold 1965). Stroh and Gerhold's theory did not address weevil behaviour during the creation of oviposition cavities, although this is probably much the same as for feeding cavities. Even though the epithelial cells of the canals are not ruptured by the weevils during excavation, the support tissues around the canals are frequently consumed which often results in shrivelling and collapsing of the resin canal, effectively deactivating it (Overhulser 1973; Alfaro 1995, Alfaro 1996b).

Trees which are resistant to the spruce weevil have been found to have a higher number of outer resin canals per square millimetre of bark than susceptible trees (Tomlin and Borden 1994, Alfaro 1996a, Alfaro *et al.* 1997). Alfaro *et al.* (1997) also found that trees attacked by *Pissodes strobi* had a lower number of both inner and outer resin canals per square millimetre than unattacked trees in the same controlled shadehouse experiment. Similarly, western white pine (*Pinus monticola* Dougl.), which is an unfavourable host for the spruce weevil, was found to have a greater number of resin canals than either eastern white pine or Engelmann spruce, both of which are acceptable hosts (Plank and Gerhold 1965, Soles *et al.* 1970).

The number of resin canals alone tells us very little about the amount of space available in the bark for feeding and oviposition because it indicates nothing about the size of the resin canals. The amount of space in a leader that is occupied by resin canals reduces the amount of space available for feeding, egg-laying and larval development. There is also a greater likelihood of an adult weevil contacting a resin duct during the excavation of a feeding or oviposition cavity. It would seem more appropriate, therefore, to examine the ratio of resin canal area to bark area. This characteristic was measured by Alfaro (1996a), who determined that resistant interior spruce trees had a greater ratio of resin canal area to bark area in the leader than susceptible trees. Alfaro only had two resistant families available in his study, and replication was low, thus confirmation of these findings for a larger sample size is needed.

Another factor that would influence the amount of space available for oviposition in a leader would be the thickness of the bark. Sullivan (1961) and Wilkinson (1983) have observed that *P. strobi* prefer leaders with thicker bark in eastern white pine. Sullivan (1961) indicated that, given the average size of 0.8 x 0.5 mm for a weevil egg, leader bark that is thinner than 0.8 mm is probably not suitable for oviposition. Thin bark appears to have an adverse affect on larval development as well as oviposition. Wallace and Sullivan (1985) demonstrated that there is a limit to the number of weevil larvae of each instar that can fit within the bark of a leader of a given diameter. This is because there must be enough room for the head capsules of all larvae within the bark as they grow and moult. This is coupled with the fact that the larvae must avoid contact

with cortical resin ducts to avoid drowning in pitch. Thus, leaders with thin bark probably would be less able to support large broods of weevils, and the production of large broods is believed to be one of the requirements for a successful weevil attack (Plank and Gerhold 1965, Alfaro 1996b).

In conifers that do not have a constitutive resin system, or in cases where insects survive this preformed defense, an induced reaction may be more important (Berryman 1972, 1988). Induced defense responses of trees are likely to be most effective against insects that have low mobility or are surrounded by plant tissues. Trees would have a better chance of developing specific defense responses to these type of insects because they likely share a close ecological and evolutionary relationship (Herms and Mattson 1992). Despite the fact that adult weevils are mobile and have the potential to avoid wound resin, eggs and tunnelling larvae do not. Young larvae also seem to be more susceptible to resin toxicity than mature larvae.

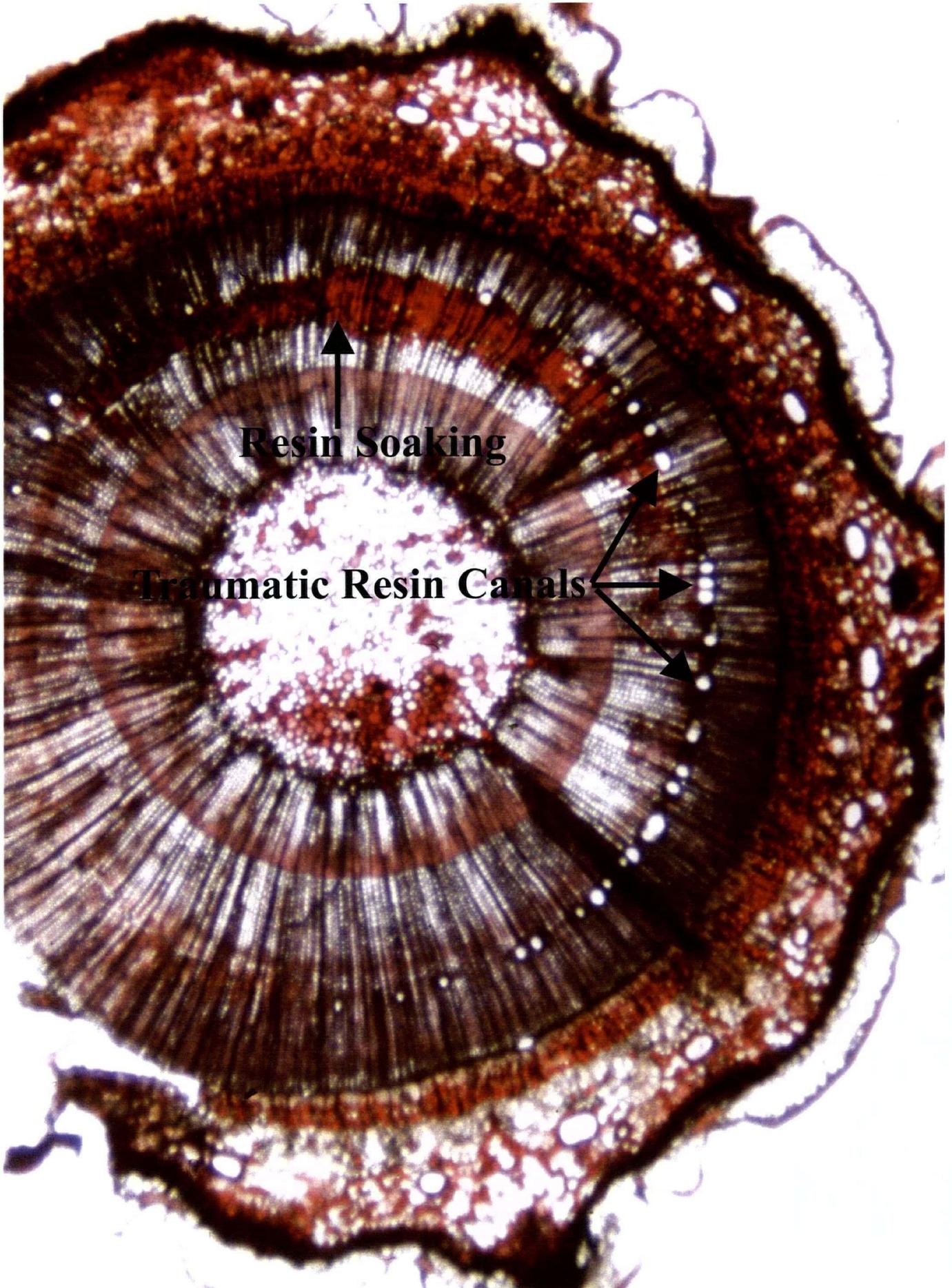
Alfaro (1995) observed that, following attack by the weevil, the cambium of an attacked white spruce tree switched from the production of normal xylem cells to form a ring of traumatic resin canals. This response was observed to have killed unhatched weevil eggs in some instances, and to have drowned larvae in others. Resin produced by the induced response has a different chemical composition than constitutive resin. It contains higher levels of toxic phenolics and terpenes (Shrimpton and Whitney 1968), and a higher monoterpene to resin acid ratio which results in it being more fluid than constitutive resin (Connor-Antonijevic 1996).

The production of traumatic resin canals was found to be distributed around the full circumference of the xylem, rather than localized at the point of injury. A leader which survived the attack would then revert back to the production of normal xylem cells. Normal xylem resin canals occur singly in spruce, however, traumatic resin canals occur adjacent to each other in rings or in portions of rings and extend vertically and radially through the xylem (Figure 3.2) (Thomson and Sifton 1926, Esau 1965, Leney and Moore 1977).

The process of traumatic resin canal formation is schizogenous (Jou 1971, Wu and Hu 1997) and has been described as follows for Japanese hemlock, *Tsuga sieboldii* (Carr.) (Kuroda and Shimaji 1983). During the first three days after wounding, groups of large rounded cells are formed in the zone of xylem mother cells. Within six days after wounding, intercellular spaces begin to form in the middles of these groups of cells. The cells surrounding these gaps then differentiate into the epithelial cells. Next, the gaps enlarge and thin-walled cells containing resin-like droplets surround the epithelium. This final stage was observed to occur approximately 20 days after wounding in both grand fir and Japanese hemlock (Wong and Berryman 1977, Kuroda and Shimaji 1983).

In white spruce, the traumatic response frequently consists of one or more layers of traumatic resin canals arranged around the circumference of the xylem (Alfaro *et al.* 1996b). The origin of these different layers is not clear. However, they are believed to be responses to different stages in the weevil attack process such as adult feeding, oviposition, and larval feeding (Alfaro *et al.* 1996b).

Figure 3.2. Cross section of an artificially wounded Sitka spruce lateral showing resin soaking and traumatic resin canals (125X).



Resin Soaking

Traumatic Resin Canals

41a

Alfaro *et al.* (1996b) observed that resistant interior spruce trees produced a higher traumatic resin response compared to susceptible trees, which suggests that traumatic resin production may be a major component of resistance in white spruce. However, they indicate that these results should be interpreted with caution because only seven resistant trees were attacked by weevils in that study. Working with white spruce, Tomlin *et al.* (1998) confirmed these findings using artificially wounded trees which allowed for a larger sample size. The wounded leaders, however, were not collected until the end of the growing season. Using Sitka spruce, Tomlin (1996) observed no difference in the induced response of resistant and susceptible clones in the field, however, only one susceptible clone was used in the study and the attacked internodes were not collected until four years after the attack had occurred. It must be noted that the traumatic resin response develops only in response to wounding (by weevils or mechanical means). Therefore, trees in which repellency plays a role in resistance may not show a traumatic wound response.

In order to properly understand how the induced response of resistant and susceptible trees differs, the factors affecting the response following wounding must be understood. Because the effectiveness of the induced response is a combination of timing and intensity (Berryman and Ashraf 1970), it is probable that the induced response of resistant trees will reach a higher intensity at an earlier time than susceptible trees.

Optimal defense theory assumes that trees which are likely to be attacked by herbivores will have high levels of constitutive defenses and little capacity for

induced defenses, while trees that are not likely to be attacked will have low levels of constitutive defense and a large capacity for induced defenses (Zangerl and Rutledge 1996). Constitutive defenses utilize plant resources on a continual basis, but can offset the cost of a delayed induced defense response. The costs of induced defenses, on the other hand, are incurred only when required, but those costs could be great if a induced response is either intense or sustained (Matson and Hain 1983, Herms and Mattson 1992, Zangerl and Rutledge 1996). Thus, it is implied that there is trade-off between constitutive and induced defenses. Tomlin *et al.* (1998), however, found no correlation between cortical resin canal density and traumatic resin production in white spruce and suggested that they are separate resistance mechanisms.

Herms and Mattson (1992) suggested that when a plant devotes resources to structural and chemical defenses, growth is decreased because resources are diverted from leaf-area production and the production of other vegetative structures, for the manufacture of defensive compounds. Thus, trade-offs must occur between growth and defense due to the limited resources available to plants. Evidence of this tradeoff has been found by Tomlin *et al.* (1998) in the case of the white pine weevil, who observed that growth was significantly reduced in white spruce trees that had been wounded compared to trees that had not been wounded. Similarly, Nebeker *et al.* (1995) found that diseased lodgepole pine (*Pinus contorta*) trees had high resin flow and low height growth and radial growth, while asymptomatic trees had low resin flow and higher height growth and radial growth.

Alfaro *et al.* (1997) found that interior spruce tree height and constitutive resin canal measurements were positively correlated, and suggested that tall or fast growing trees have a better developed resin canal system, and are therefore more resistant to *P. strobi* attack. In both loblolly pine and grand fir (*Abies grandis* (Dougl.) Lindl.), resin flow was found to be positively correlated with tree vigour (Matson *et al.* 1987, Filip *et al.* 1989).

The objectives of this study were to test the hypotheses that: 1) Sitka spruce trees resistant to the white pine weevil have a higher ratio of cortical resin canal area to bark area than susceptible trees; 2) resistant trees have thinner bark than susceptible trees; 3) there is a tradeoff between constitutive and induced defenses in Sitka spruce; 4) there is a tradeoff between defense and growth in Sitka spruce; 5) resistant trees will produce a greater traumatic response earlier in the season than susceptible trees.

3.2 MATERIAL AND METHODS

3.2.1 Study Site and Sample Collection

This study was conducted from March to August 1998 at the Jordan River and Cowichan Lake study sites described in Chapter 2. Three trees were randomly selected from each of the same four weevil-resistant families (No.'s 12, 17, 24, 120) and four susceptible families (3, 11, 41, 99) from the previous experiment. On each tree, four pairs of laterals (8 branches) were selected from a mid-crown whorl. The pairs were randomly numbered from one to four to be collected on one of four different dates.

One branch of each pair, selected randomly, was wounded using a Dremmel® drill with a one millimetre bit and the other was left as a control. Twenty-four holes spaced 0.5 cm apart were drilled along the length of the lateral starting two centimetres below the apical bud. The holes were made in three lines (eight holes each) around the circumference of the branch and penetrated the bark only. Wounding took place on April 30, 1998 at Jordan River and on May 1 at Cowichan Lake and coincided with the natural weevil attack period. Laterals were collected while the trees were quiescent (Mar 28-Jordan R., Mar 29-Cowichan Lk.), shortly after wounding (May 14-Jordan R., May 13-Cowichan Lk.), later in the season (Jul 6-Jordan R., Jul 7- Cowichan Lk.), and at the end of the season (Aug 26-Jordan R., Aug 27-Cowichan Lk.).

Samples were immediately brought to the laboratory and a five cm section was taken from the distal end of each lateral and fixed in FAA (formalin - alcohol - acetic acid) (Johansen 1940). Cross-sections 60 µm thick were made using a sliding microtome, then stained in a 1% aqueous Safranin O solution for microscopic observations of traumatic resin canal formation and videocapture of microscopic images.

3.2.2 Data Collection

The cross-sections were scanned using the Sigma Scan® Image Analysis System. The following information was collected: total bark area, number and area of both inner and outer bark resin canals, radius from the pith to the outer edge of the bark, radius from the pith to the first growth ring, radius from the pith

to the second growth ring, radius from the pith to the inner edge of the ring of traumatic resin canals (if present). The proportion of bark that was occupied by inner and outer resin canals was estimated from the above information, as was the number of resin canals per square micron of bark. The mean size of both inner and outer resin canals was also estimated.

Based on microscopic observations, the induced resin response was categorized from 0-4 according to intensity. A score of zero indicated no response (Figure 3.3a), a score of 1 indicated the presence of resin soaking in the xylem but no formation of traumatic resin canals (Figure 3.3b), 2 indicated the presence of scattered resin canals (Figure 3.3c), 3 indicated an incomplete or discontinuous ring of resin canals (Figure 3.3d), and a 4 indicated a complete or continuous ring of traumatic resin canals (Figure 3.3e).

3.2.3 Data Analyses

Several variables were analyzed, with a repeated measures ANOVA using the same model as described in Section 2.2.3 ($Y_{ijk} = \mu + R_i + S_j + RS_{ij} + \epsilon_{(ij)k}$ where R and S were fixed and R represented the resistance or wounding status of a tree depending on the test being applied), to determine if they changed through time, and if they differed between trees from resistant and susceptible families or between wounded and unwounded trees. These variables included the number of inner, outer, and total resin canals per square micron of bark, the percentage of bark occupied by inner, outer, and total resin canals (ratio of resin canal area to bark area), the mean inner and outer resin canal sizes, the width of the first

Figure 3.3a. Cross section of Sitka spruce lateral showing a Type 0 traumatic resin response (no response) to artificial wounding with 24 holes (12.5X).



47a

Figure 3.3b. Cross section of Sitka spruce lateral showing a Type 1 traumatic resin response (resin soaking) to artificial wounding with 24 holes (12.5X).



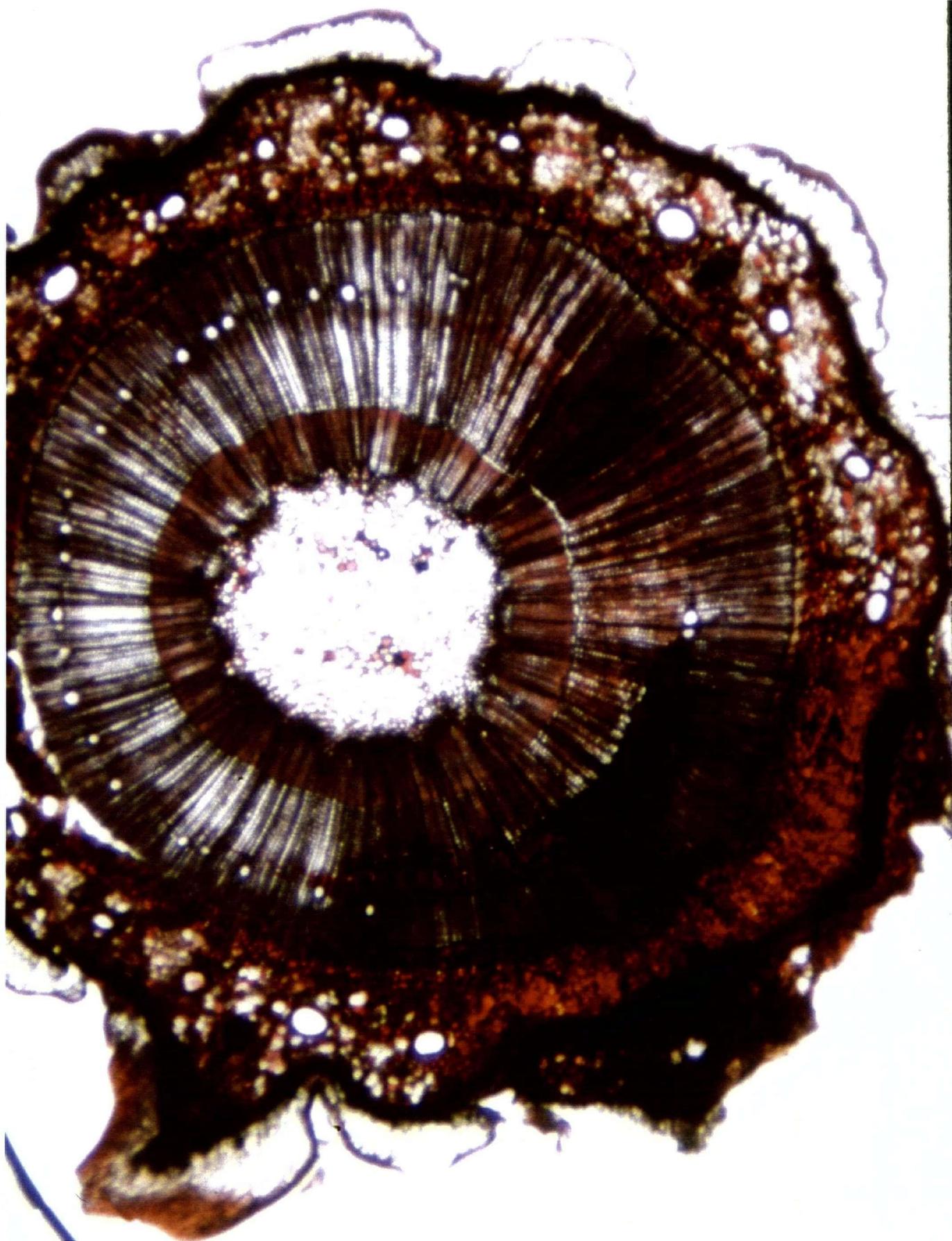
48a

Figure 3.3c. Cross section of Sitka spruce lateral showing a Type 2 traumatic resin response (scattered resin canals) to artificial wounding with 24 holes (12.5X).



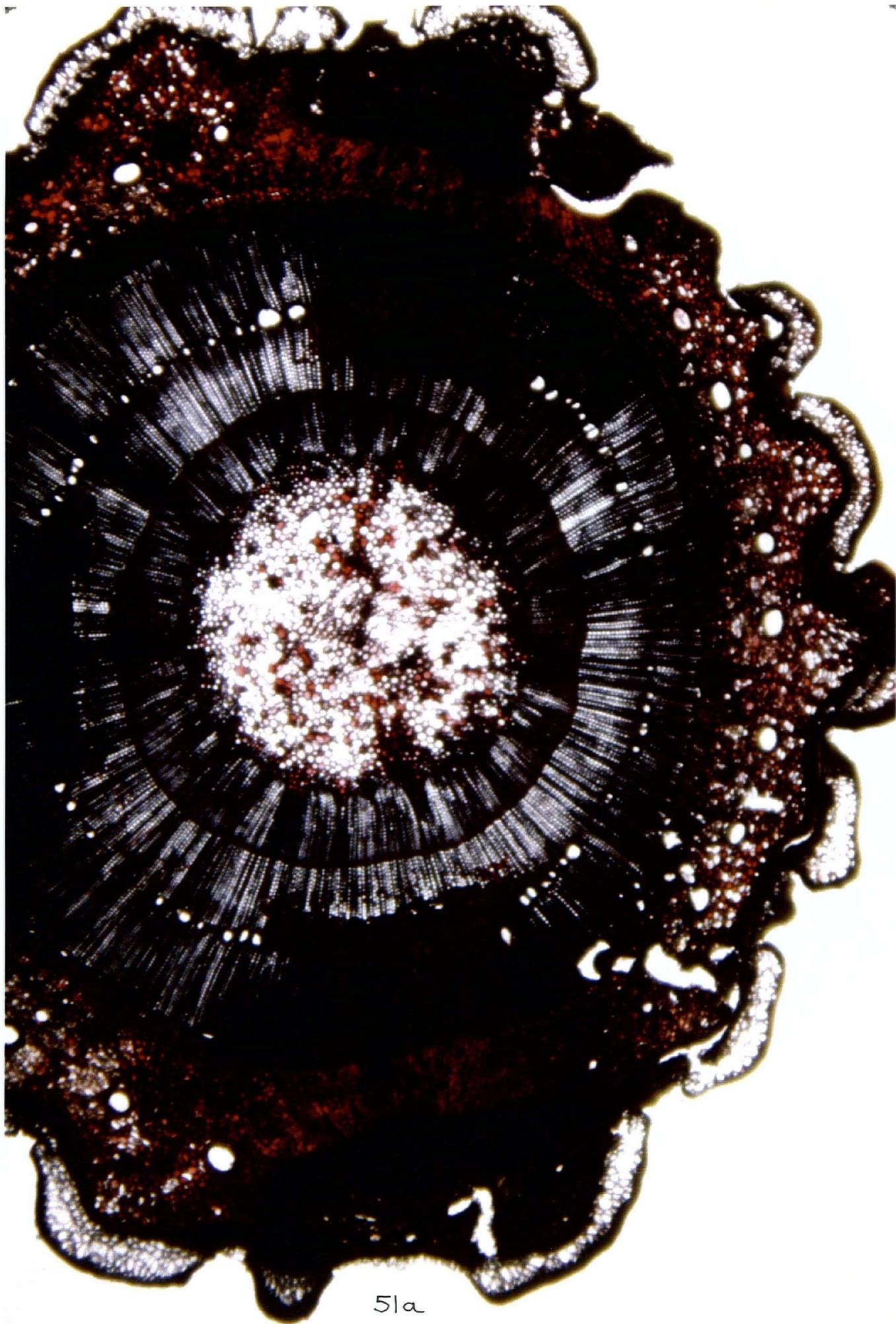
49a

Figure 3.3d. Cross section of Sitka spruce lateral showing a Type 3 traumatic resin response (incomplete ring of traumatic resin canals) to artificial wounding with 24 holes (12.5X).



50a

Figure 3.3e. Cross section of Sitka spruce lateral showing a Type 4 traumatic resin response (complete ring of traumatic resin canals) to artificial wounding with 24 holes (12.5X).



51a

and of the second growth rings, and the mean traumatic response code. Non-normal variables were transformed using the Square root transformation or the Arcsine transformation where appropriate (Zar 1996). Analysis of variance was used to determine differences between resistant and susceptible trees or wounded and unwounded trees at particular sampling dates, and Tukey's test was used to compare mean inner resin canal size at different sampling dates (Zar 1996). Pearson product-moment correlations (Zar 1996) were used to look for possible relationships (tradeoffs are negative correlations) between leader variables in trees from resistant families and susceptible families and the variables measuring resin canal density and traumatic resin production.

3.3 RESULTS

3.3.1 Density of Resin Canals

In this study, the density of resin canals was defined as the number of resin canals per square micron of bark. The number of outer resin canals per square micron of bark was not normally distributed; however, when a square root transformation was done, there was no significant change in the normality of the data. These findings were also true when cube root or fourth root transformations were done. As previously mentioned in Section 2.3, Zar (1996) states that analysis of variance is robust with respect to deviations from normality.

When examining the number of cortical resin canals per square micron of bark, no significant difference was found between trees from resistant and

susceptible families in inner resin canals ($F_{(1,86)}=0.42$, $p=0.52$), outer resin canals ($F_{(1,86)}=1.00$, $p=0.32$), or total resin canals ($F_{(1,86)}=0.17$, $p=0.68$) through the season (Table 3.1a). This was also true when each study site was examined separately. When looking at the first measurement period alone, however, the trees from resistant families did have a significantly higher density of inner resin canals than the trees from susceptible families ($F_{(1,94)}=4.35$, $p=0.04$) (Figure 3.4). No difference was found in the number inner ($F_{(1,86)}=2.18$, $p=0.14$), outer ($F_{(1,86)}=0.63$, $p=0.43$), or total resin canals ($F_{(1,86)}=0.09$, $p=0.76$) between wounded and unwounded trees (Table 3.1b). Both the density of inner resin canals ($F_{(3,258)}=34.34$, $p=0.00$) and outer resin canals ($F_{(3,258)}=14.66$, $p=0.00$) (number per square micron of bark) were found to decrease over time, as a result of the increasing internode circumference (Figures 3.4 and 3.5).

3.3.2 Percentage of Bark Occupied by Resin Canals

In this study, the percentage of bark occupied by resin canals was defined as the total area of resin canals (in square microns) per square micron of bark. The percentage of bark occupied by both inner and outer resin canals was not normally distributed at all sample times; however, when an arcsine transformation was done, no significant change in normality of the data was observed. The overall effect on the analysis, however, is minimal, as mentioned in Section 3.3.1 above.

Table 3.1a. Mean number of inner, outer, and total resin canals per square millimetre of Sitka spruce bark for weevil resistant and susceptible trees, pooled for both wounded and unwounded trees and both sites, at four different sampling times during the season. Sample times at which resistant and susceptible trees are significantly different from each other ($p < 0.05$), for a given canal type, are denoted by different letters.

	Inner Resistant	Inner Susceptible	Outer Resistant	Outer Susceptible	Total Resistant	Total Susceptible
Mar 28/29						
N	48	48	48	48	48	48
Mean (No./mm ²)	2.36 ^a	2.07 ^b	0.72 ^f	1.02 ^f	3.08 ^w	3.11 ^w
Std. Error of Mean	0.12	0.090	0.12	0.13	0.16	0.14
May 14/15						
N	48	49	48	49	48	49
Mean (No./mm ²)	2.05 ^c	1.98 ^c	0.88 ^g	0.90 ^g	2.93 ^x	2.89 ^x
Std. Error of Mean	0.090	0.090	0.12	0.11	0.13	0.14
Jul 6/7						
N	46	48	46	48	46	48
Mean (No./mm ²)	1.67 ^d	1.65 ^d	0.61 ^h	0.61 ^h	2.28 ^y	2.26 ^y
Std. Error of Mean	0.076	0.10	0.094	0.092	0.13	0.13
Aug 25/26						
N	44	46	44	46	44	46
Mean (No./mm ²)	1.51 ^e	1.61 ^e	0.46 ⁱ	0.52 ⁱ	1.97 ^z	2.13 ^z
Std. Error of Mean	0.085	0.093	0.081	0.086	0.10	0.13

Table 3.1b. Mean number of inner, outer, and total resin canals per square millimetre of Sitka spruce bark for wounded and unwounded trees, pooled for both weevil resistant and susceptible trees and both sites, at four different sampling times during the season. Sample times at which wounded and unwounded trees are significantly different from each other ($p < 0.05$), for a given canal type, are denoted by different letters.

	Inner Unwounded	Inner Wounded	Outer Unwounded	Outer Wounded	Total Unwounded	Total Wounded
Mar 28/29						
N	48	48	48	48	48	48
Mean (No./mm ²)	2.24 ^a	2.20 ^a	0.87 ^f	0.87 ^f	3.12 ^w	3.07 ^w
Std. Error of Mean	0.088	0.11	0.12	0.13	0.14	0.15
May 14/15						
N	48	49	48	49	48	49
Mean (No./mm ²)	2.00 ^b	2.04 ^b	0.80 ^g	0.97 ^g	2.81 ^x	3.01 ^x
Std. Error of Mean	0.098	0.082	0.12	0.12	0.13	0.14
Jul 6/7						
N	47	47	47	47	47	47
Mean (No./mm ²)	1.78 ^c	1.54 ^c	0.55 ^h	0.67 ^h	2.32 ^y	2.21 ^y
Std. Error of Mean	0.095	0.082	0.081	0.10	0.12	0.13
Aug 25/26						
N	45	45	45	45	45	45
Mean (No./mm ²)	1.75 ^d	1.38 ^e	0.49 ⁱ	0.50 ⁱ	2.24 ^z	1.87 ^z
Std. Error of Mean	0.098	0.069	0.095	0.072	0.13	0.10

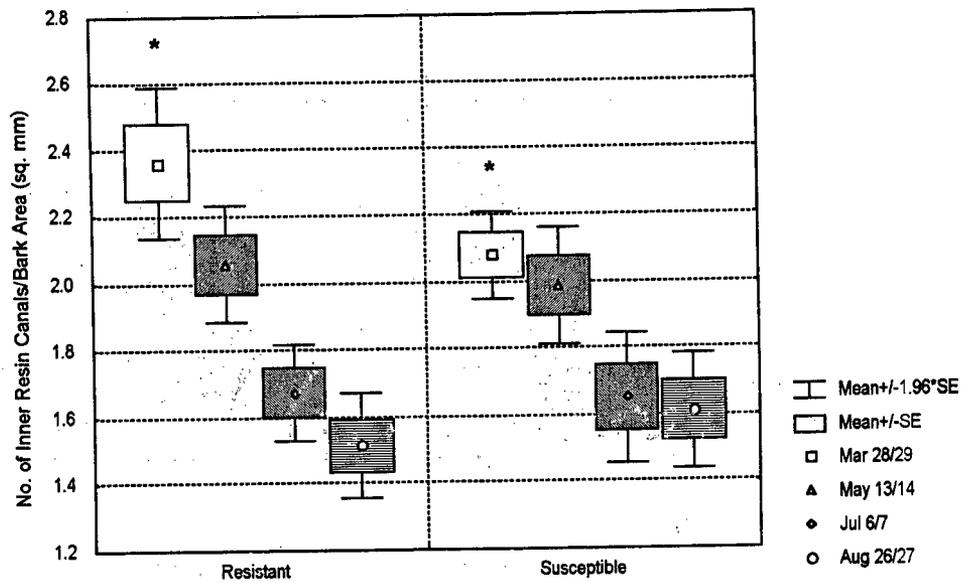


Figure 3.4. Mean number of inner resin canals per square millimetre of Sitka spruce bark for weevil resistant and susceptible trees (both wounded and unwounded) at four times during the year for Jordan River and Cowichan Lake combined. * Indicates differences are significant at the 0.05 level of probability.

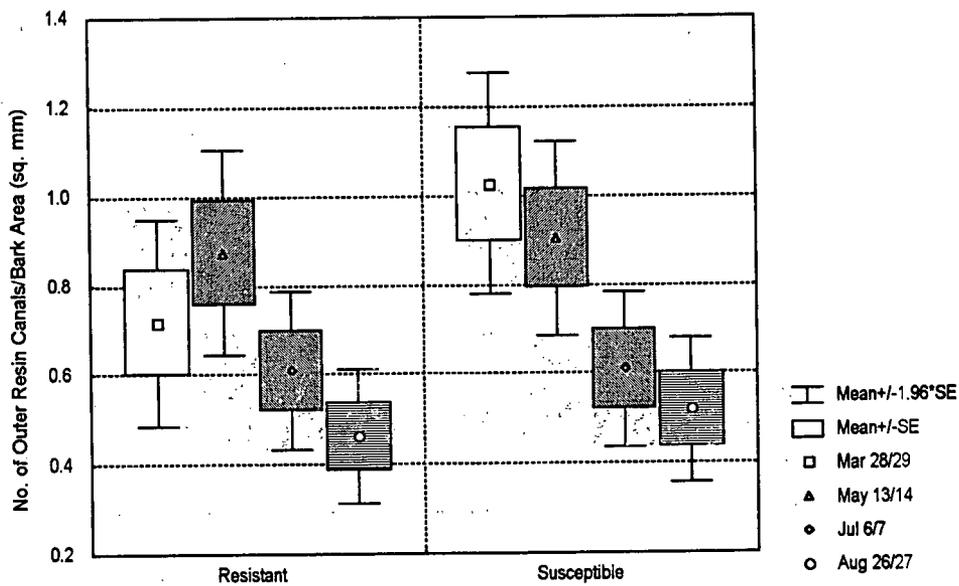


Figure 3.5. Mean number of outer resin canals per square millimetre of Sitka spruce bark for weevil resistant and susceptible trees (both wounded and unwounded) at four times during the year for Jordan River and Cowichan Lake combined.

3.3.2.1 Percentage of Bark Occupied by Inner Resin Canals

Using the pooled data for both sites, no detectable difference was found between trees from resistant and susceptible families for the percentage of bark occupied by inner resin canals ($F_{(1,86)}=1.81$, $p=0.18$) (Table 3.2). However, when examining the Jordan River site alone, trees from resistant families were found to have a significantly larger percentage of bark occupied by inner canals over the four measurement periods ($F_{(1,38)}=8.54$, $p=0.006$) (Figure 3.6, Table 3.2). The Cowichan Lake site showed no significant difference between the two resistance categories for the percentage of bark occupied by inner resin canals ($F_{(1,46)}=0.18$, $p=0.68$) (Table 3.2).

No significant difference in the percentage of bark occupied by inner resin canals was found between wounded and unwounded trees ($F_{(1,86)}=0.93$, $p=0.34$). Pooling wounded and unwounded samples, the percentage of bark occupied by inner resin canals did decline over the first three measurement dates, but there was an increase on the fourth measurement date (Figure 3.7) (Table 3.2) that appears to correspond with the increase in resin canal size at the end of the summer ($F_{(3,261)}=9.20$, $p=0.00$) (Figure 3.9a) (Table 3.3).

3.3.2.2 Percentage of Bark Occupied by Outer Resin Canals

No difference was found between the trees from resistant and susceptible families for the outer resin canals at Jordan River ($F_{(1,38)}=0.24$, $p=0.63$), Cowichan Lake ($F_{(1,46)}=0.24$, $p=0.88$), or both sites combined ($F_{(1,86)}=0.03$, $p=0.86$) (Table 3.4). Although there was no difference between trees from

Table 3.2. Mean percentage of Sitka spruce bark occupied by inner resin canals for weevil resistant and susceptible trees, pooled for both wounded and unwounded trees, at four different sampling times during the season. Sample times at which resistant and susceptible trees are significantly different from each other ($p < 0.05$) for a given site, are denoted by different letters.

	Jordan Resistant	Jordan Susceptible	Cowichan Resistant	Cowichan Susceptible	BothSites Resistant	BothSites Susceptible
Mar 28/29						
N	24	24	24	24	48	48
Mean Percent	0.96 ^a	0.78 ^b	1.10 ^h	1.10 ^h	1.10 ^w	0.96 ^w
Std. Error of Mean	0.050	0.046	0.078	0.065	0.048	0.047
May 14/15						
N	24	25	24	24	48	49
Mean Percent	0.77 ^c	0.60 ^d	1.10 ⁱ	1.10 ⁱ	0.92 ^x	0.81 ^x
Std. Error of Mean	0.068	0.041	0.091	0.066	0.060	0.049
Jul 6/7						
N	22	24	24	24	46	48
Mean Percent	0.70 ^e	0.65 ^e	0.92 ^j	0.96 ^j	0.82 ^y	0.80 ^y
Std. Error of Mean	0.049	0.050	0.069	0.12	0.046	0.066
Aug 25/26						
N	20	22	24	24	44	46
Mean Percent	0.82 ^f	0.54 ^g	1.20 ^k	1.10 ^k	1.10 ^z	0.81 ^z
Std. Error of Mean	0.12	0.032	0.19	0.093	0.12	0.064

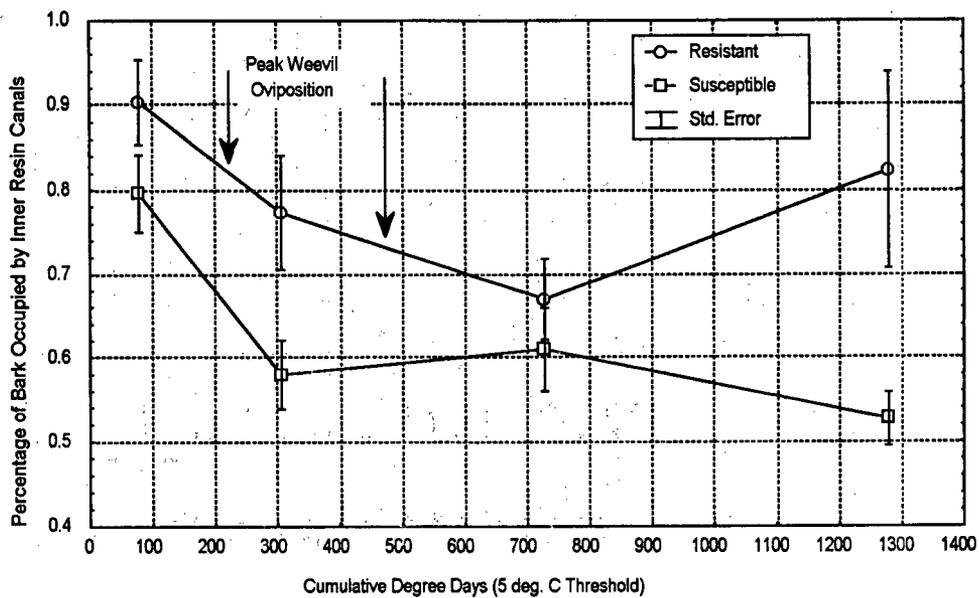


Figure 3.6. Percentage of Sitka spruce bark occupied by inner resin canals for weevil resistant and susceptible trees at Jordan river at four times during the growing season, including both wounded and unwounded treatments.

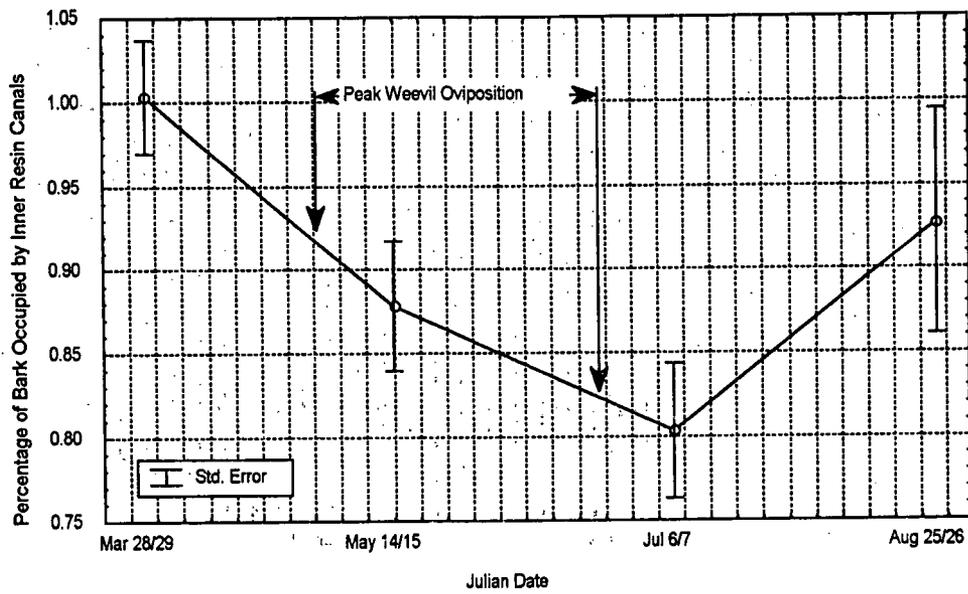


Figure 3.7. Percentage of Sitka spruce bark occupied by inner resin canals at Jordan River and Cowichan Lake combined, measured at four times during the growing season and pooled over both resistance categories.

Table 3.3. Mean cross-sectional size of inner resin canals (mm²) at four different sampling times pooled over both sites including weevil resistant and susceptible trees and wounded and unwounded trees. Sample times which are significantly different from each other (p<0.05) are denoted by different letters.

	N	Mean (mm ²)	Std. Error of Mean
Mar 28/29	96	0.0048 ^a	0.00020
May 14/15	97	0.0049 ^a	0.00032
Jul 6/7	94	0.0057 ^a	0.00039
Aug 25/26	90	0.0070 ^b	0.00061

resistant and susceptible families in the percentage of bark occupied by outer resin canals, there appears to be a slight decline over time in the mean value for this trait (Figure 3.8). No significant difference between wounded and unwounded trees was found for this trait either ($F_{(1,86)}=1.42$, $p=0.24$).

3.3.3 Resin Canal Size

The mean cross-sectional resin canal size for both inner and outer canals was not normally distributed at all sample times; however, when a square root transformation was done, there was no significant change in the normality of the data. These findings were also true when cube root or fourth root transformations were done, however the overall effect on the analysis is minimal, as mentioned in Section 3.3.1 above.

Table 3.4. Mean percentage of Sitka spruce bark occupied by outer resin canals for weevil resistant and susceptible trees, pooled for both wounded and unwounded trees, at four different sampling times during the season. Sample times at which resistant and susceptible trees are significantly different from each other ($p < 0.05$) for a given site, are denoted by different letters.

	Jordan Resistant	Jordan Susceptible	Cowichan Resistant	Cowichan Susceptible	BothSites Resistant	BothSites Susceptible
Mar 28/29						
N	24	24	24	24	48	48
Mean Percent	0.077 ^a	0.093 ^a	0.062 ^e	0.097 ^e	0.070 ^w	0.095 ^w
Std. Error of Mean	0.018	0.018	0.019	0.024	0.013	0.015
May 14/15						
N	24	25	24	24	48	49
Mean Percent	0.060 ^b	0.067 ^b	0.10 ^f	0.090 ^f	0.082 ^x	0.078 ^x
Std. Error of Mean	0.015	0.019	0.021	0.016	0.013	0.013
Jul 6/7						
N	22	24	24	24	46	48
Mean Percent	0.067 ^c	0.042 ^c	0.089 ^g	0.076 ^g	0.078 ^y	0.059 ^y
Std. Error of Mean	0.019	0.0092	0.030	0.019	0.018	0.011
Aug 25/26						
N	20	22	24	24	44	46
Mean Percent	0.083 ^d	0.047 ^d	0.061 ^h	0.065 ^h	0.071 ^z	0.057 ^z
Std. Error of Mean	0.030	0.013	0.016	0.015	0.016	0.010

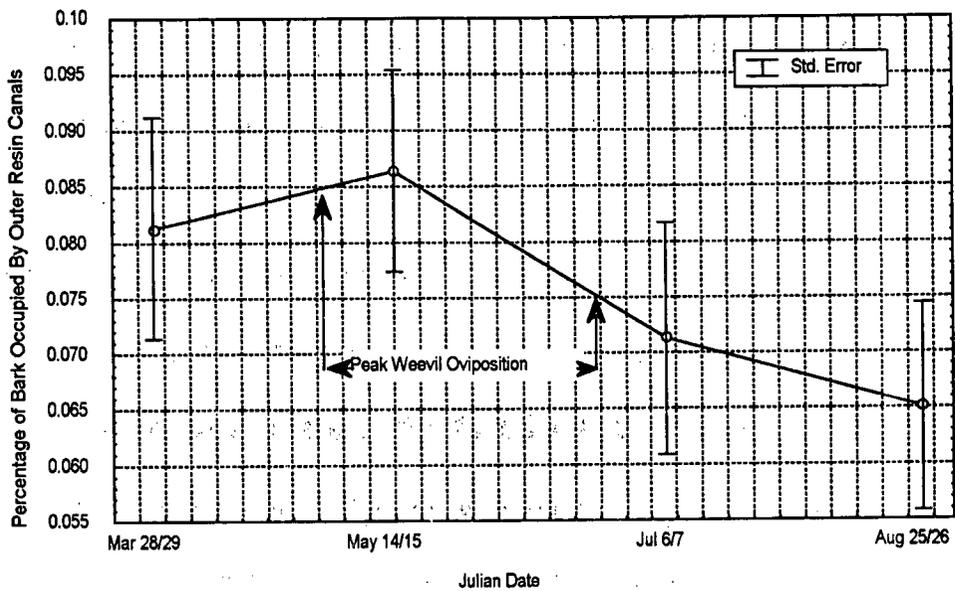


Figure 3.8. Percentage of Sitka spruce bark occupied by outer resin canals at four times during the growing season, pooled over both sites, both weevil resistance categories, and including both wounded and unwounded trees.

3.3.3.1 Inner Resin Canal Size

Inner resin canals show a significant increase in size through the season ($F_{(3,254)}=9.35, p=0.00$) and are substantially larger than outer resin canals at any given time (Figure 3.9a) (Table 3.5). Additionally, the mean size of the inner resin ducts of trees from resistant families was found to be significantly larger than the mean size of inner resin ducts in susceptible trees at the Jordan River site ($F_{(1,38)}=6.05, p=0.02$) (Figure 3.10) (Table 3.5). This difference was not evident at the Cowichan Lake site ($F_{(1,46)}=0.019, p=0.89$), or at both sites combined ($F_{(1,86)}=0.50, p=0.48$).

When comparing wounded versus unwounded laterals, no difference was found in the size of the inner resin ducts at the time of the first and second samplings, but the wounded laterals were found to have significantly larger inner resin ducts at the time of the third and fourth sampling periods (Figure 3.11).

The trees at the Cowichan Lake site had significantly larger inner resin canals than the Jordan River site ($F_{(1,86)}=40.14, p=0.00$), but had fewer inner canals per square micron of bark ($F_{(1,86)}=11.99, p=0.00$).

As the size of the inner resin ducts increases, the percentage of bark occupied by inner canals increases and the number of resin canals per square micron of bark decreases. The percentage of bark occupied by inner canals and the number of inner canals per square micron of bark were unrelated (Table 3.6).

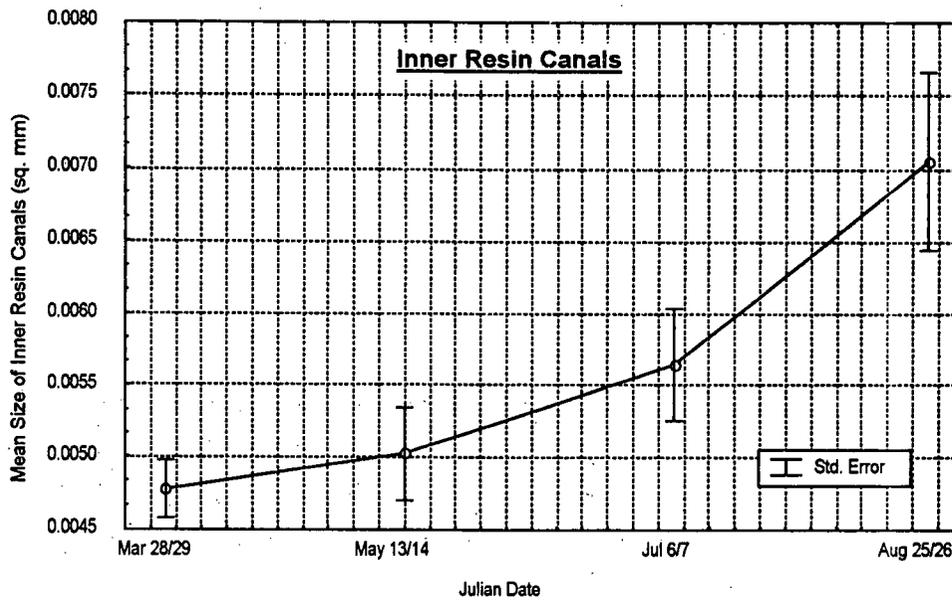


Figure 3.9a. Mean cross-sectional size of inner resin canals at four times during the growing season, pooled over both sites, both weevil resistance categories, and including both wounded and unwounded Sitka spruce trees.

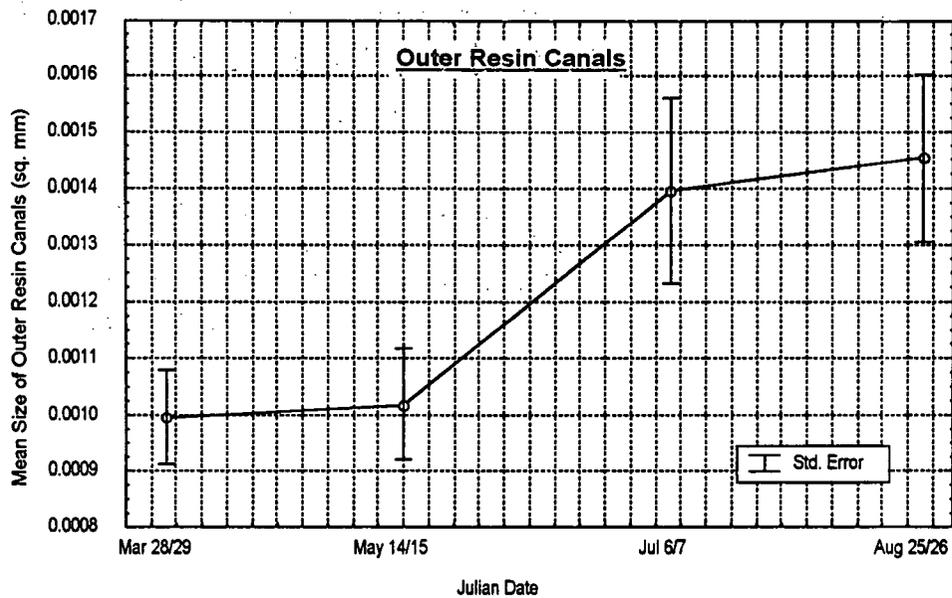


Figure 3.9b. Mean cross-sectional size of outer resin canals at four times during the growing season, pooled over both sites, both weevil resistance categories, and including both wounded and unwounded Sitka spruce trees.

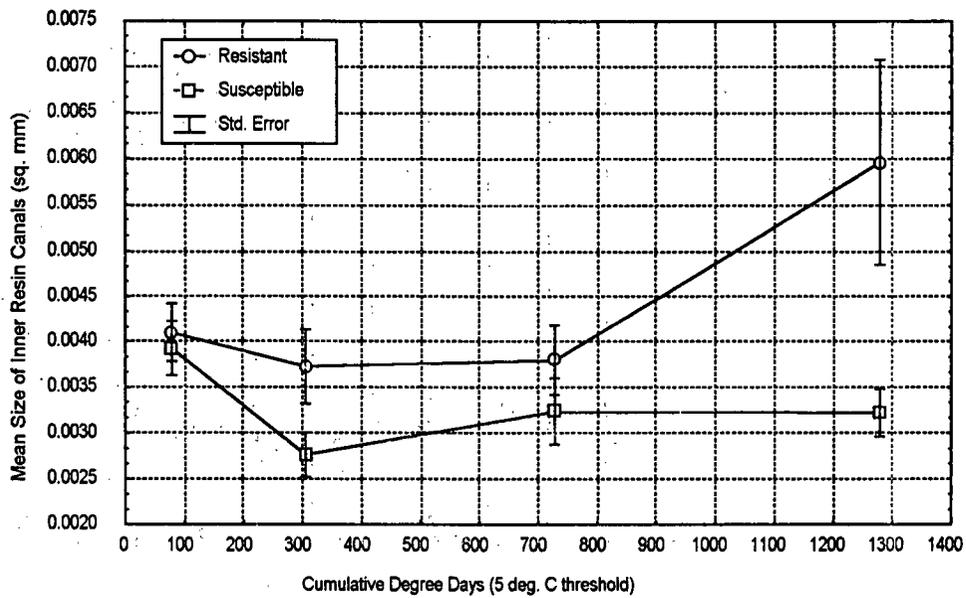


Figure 3.10. Mean cross-sectional size of inner resin canals for weevil resistant and susceptible Sitka spruce at Jordan River at four times during the growing season, including both wounded and unwounded Sitka spruce trees.

Table 3.5. Mean cross-sectional size (mm²) of inner resin canals for weevil resistant and susceptible trees, pooled for both wounded and unwounded trees, at four different sampling times during the season. Sample times at which resistant and susceptible trees are significantly different from each other (p<0.10) for a given site, are denoted by different letters.

	Jordan Resistant	Jordan Susceptible	Cowichan Resistant	Cowichan Susceptible	BothSites Resistant	BothSites Susceptible
Mar 28/29						
N	24	24	24	24	48	48
Mean Size (mm ²)	0.0044 ^a	0.0041 ^a	0.0053 ^g	0.0055 ^g	0.0049 ^w	0.0048 ^w
Std. Error of Mean	0.00032	0.00030	0.00052	0.00037	0.00031	0.00025
May 14/15						
N	24	25	24	24	48	49
Mean Size (mm ²)	0.0036 ^b	0.0029 ^c	0.0066 ^h	0.0064 ^h	0.0051 ^x	0.0046 ^x
Std. Error of Mean	0.00041	0.00025	0.00089	0.00051	0.00053	0.00038
Jul 6/7						
N	22	24	24	24	46	48
Mean Size (mm ²)	0.0041 ^d	0.0036 ^d	0.0066 ⁱ	0.0082 ⁱ	0.0054 ^y	0.0059 ^y
Std. Error of Mean	0.00038	0.00036	0.00063	0.0011	0.00042	0.00065
Aug 25/26						
N	20	22	24	24	44	46
Mean Size (mm ²)	0.0060 ^e	0.0032 ^f	0.0096 ^j	0.0086 ^j	0.0080 ^z	0.0060 ^z
Std. Error of Mean	0.0011	0.00026	0.0016	0.00089	0.0011	0.00062

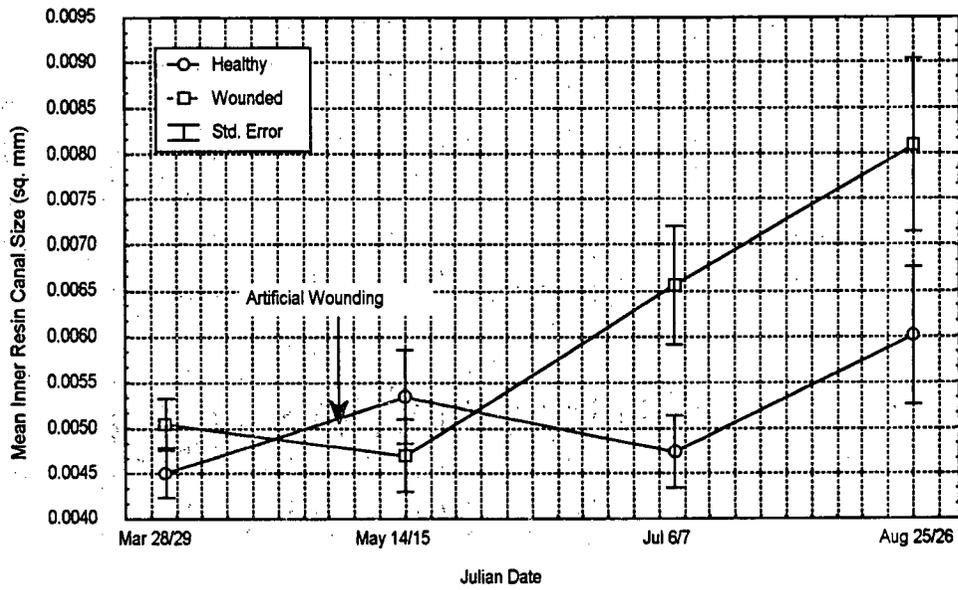


Figure 3.11. Mean cross-sectional size of inner resin canals for wounded and unwounded Sitka spruce trees at four times during the season, pooled over both sites and both weevil resistance categories. (Wounding occurred on May 1.)

Table 3.6. Correlation coefficients and levels of significance for correlations between inner resin canal size, percentage of bark occupied by inner resin canals, and number of inner resin canals per square micron of bark at four times during the growing season.

	Mar 28/29	May 14/15	Jul 6/7	Aug 25/26
Inner Canal Size vs. % Bark Occupied by Canals	0.78 p=0.00	0.79 p=0.00	0.79 p=0.00	0.89 p=0.00
Inner Canal Size vs. No. of Canals/ μm^2 of Bark	-0.56 p=0.00	-0.60 p=0.00	-0.57 p=0.00	-0.48 p=0.00
No. of Canals/ μm^2 of Bark vs. % Bark Occupied by Canals	0.0006 p=0.99	-0.16 p=0.15	-0.12 p=0.28	-0.10 p=0.35

3.3.3.2 Outer Resin Canal Size

The mean size of the outer resin ducts also showed an increase over time (Figure 3.9b), but no overall difference was found between the mean size of these ducts for trees from resistant and susceptible families at either Cowichan Lake ($F_{(1,17)}=0.02$, $p=0.87$) or at both sites combined ($F_{(1,37)}=1.34$, $p=0.25$) (Table 3.7). At Jordan River, however, resistant trees appeared to have larger outer resin canals than susceptible trees. Over all four sampling dates, this was only significant at the 0.11 level of probability ($F_{(1,18)}=2.80$), but this was significant at the 0.05 level of probability over the last 2 sampling dates (Figure 3.12). When looking at wounded trees alone, the trees from resistant families show a greater increase than trees from susceptible families in resin duct size following the time of wounding ($F_{(1,24)}=3.45$, $p=0.07$ for T2-4 and $F_{(1,25)}=4.93$, $p=0.04$ for T3-4) (Figure 3.13a). Unwounded trees show no difference in the rate of size increase

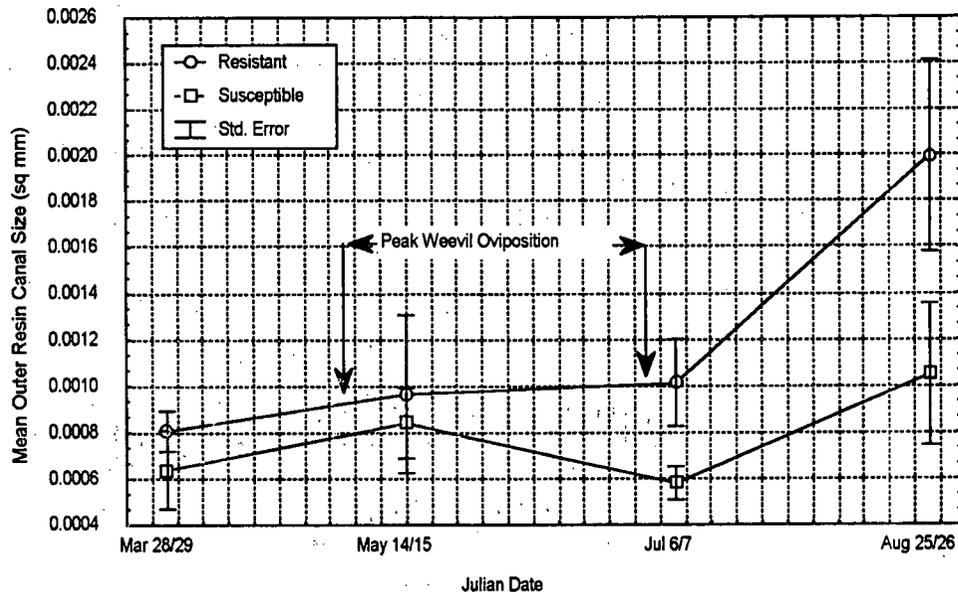


Figure 3.12. Mean cross-sectional size of outer resin canals for weevil resistant and susceptible Sitka spruce at Jordan River at four times during the growing season, including both wounded and unwounded Sitka spruce trees.

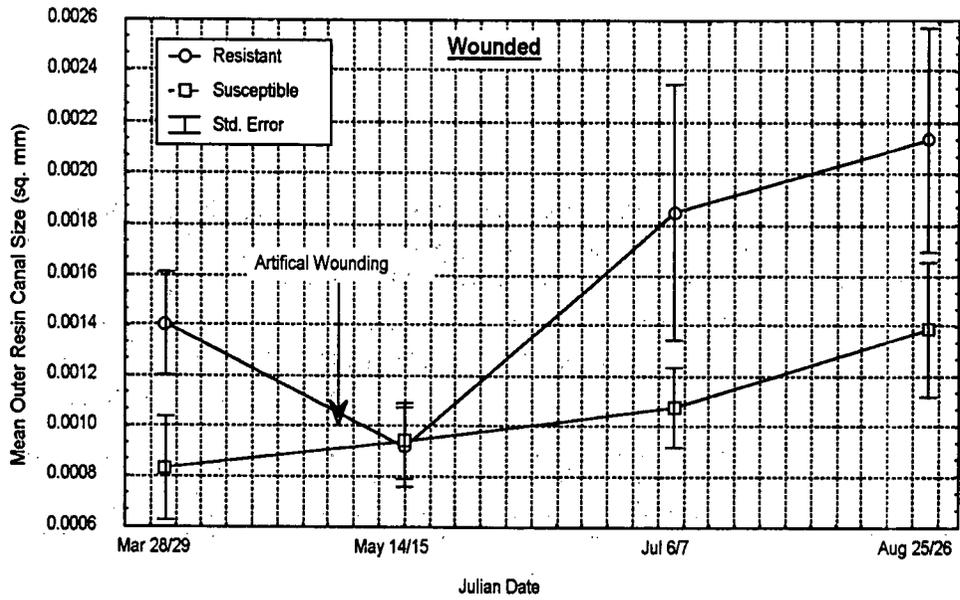


Figure 3.13a. Mean cross-sectional size of outer resin canals for weevil resistant and susceptible Sitka spruce trees at four times during the season (wounded trees only), pooled over both sites.

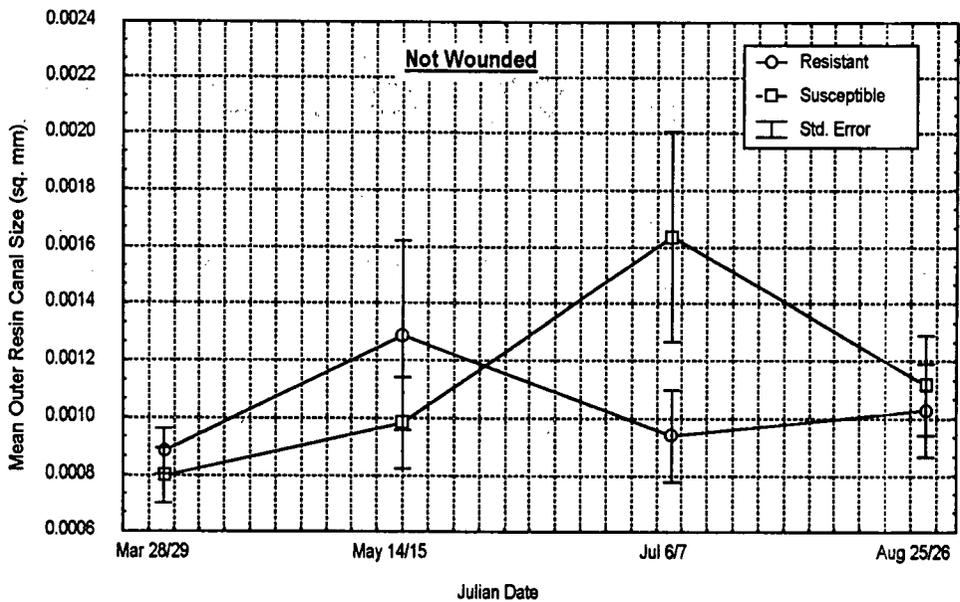


Figure 3.13b. Mean cross-sectional size of outer resin canals for weevil resistant and susceptible Sitka spruce trees at four times during the season (unwounded trees only), pooled over both sites.

Table 3.7. Mean cross-sectional size (mm^2) of outer resin canals for weevil resistant and susceptible trees, pooled for both wounded and unwounded trees, at four different sampling times during the season. Sample times at which resistant and susceptible trees are significantly different from each other ($p < 0.10$) for a given site, are denoted by different letters.

	Jordan Resistant	Jordan Susceptible	Cowichan Resistant	Cowichan Susceptible	BothSites Resistant	BothSites Susceptible
Mar 28/29						
N	19	19	12	16	31	35
Mean Size (mm^2)	0.00089 ^a	0.00089 ^a	0.0013 ^g	0.0010 ^g	0.0010 ^w	0.00095 ^w
Std. Error of Mean	0.000086	0.00017	0.00028	0.00016	0.00012	0.00012
May 14/15						
N	13	19	18	18	31	37
Mean Size (mm^2)	0.0011 ^b	0.00080 ^b	0.0011 ^h	0.0011 ^h	0.0011 ^x	0.00094 ^x
Std. Error of Mean	0.00034	0.00015	0.00018	0.00015	0.00017	0.00011
Jul 6/7						
N	13	17	17	16	30	33
Mean Size (mm^2)	0.0010 ^c	0.00063 ^d	0.0015 ⁱ	0.0018 ⁱ	0.0013 ^y	0.0012 ^y
Std. Error of Mean	0.00019	0.000073	0.00046	0.00034	0.00027	0.00020
Aug 25/26						
N	11	15	15	18	26	33
Mean Size (mm^2)	0.0020 ^e	0.00099 ^f	0.0013 ^j	0.0014 ^j	0.0016 ^z	0.0012 ^z
Std. Error of Mean	0.00042	0.00030	0.00031	0.00018	0.00025	0.00017

over the growing season between resistant and susceptible trees ($F_{(1,17)}=0.46$, $p=0.51$) (Figure 3.13b).

A significant positive correlation was found between the size of the outer resin ducts and the percentage of bark occupied by outer resin ducts, as well as between the number of resin canals per square micron of bark and the percentage of bark occupied by outer resin canals (Table 3.8). Similarly to inner resin canals, a negative correlation was found between outer resin canal size and the number of resin canals per square micron of bark, but this correlation was found only at the second and third sampling times. A positive correlation was found between inner resin canal size and outer resin canal size for the first three sampling periods.

Table 3.8. Correlation coefficients and levels of significance for correlations between outer resin canal size, percentage of bark occupied by outer resin canals, number of outer resin canals per square micron of bark, and inner resin canal size at four times during the growing season.

	Mar 28/29	May 14/15	Jul 6/7	Aug 25/26
Outer Canal Size vs. % Bark Occupied by Canals	0.63 $p<0.01$	0.82 $p<0.01$	0.82 $p<0.01$	0.78 $p<0.01$
Outer Canal Size vs. No. of Canals/ μm^2 of Bark	-0.12 $p=0.47$	-0.33 $p=0.04$	-0.32 $p=0.05$	-0.17 $p=0.31$
No. of Canals/ μm^2 of Bark vs. % Bark Occupied by Canals	0.81 $p<0.01$	0.58 $p<0.01$	0.50 $p<0.01$	-0.61 $p<0.01$
Outer Canal Size vs. Inner Canal Size	0.43 $p=0.007$	0.61 $p<0.01$	0.40 $p=0.01$	0.20 $p=0.23$

3.3.4 Bark Thickness

Trees from resistant and susceptible families both showed the same rate of increase in bark thickness over the growing season, but resistant trees had significantly thinner bark during the first three sampling periods ($F_{(1,91)}=5.91$, $p=0.02$) (Figure 3.14). The bark of trees that had been wounded showed an increase over time ($F_{(3,126)}=4.05$, $p=0.009$), but trees that had not been wounded showed no significant increase in bark thickness over time (Figure 3.15). Figure 3.16 shows the change in bark thickness for wounded resistant, wounded susceptible, unwounded resistant, and unwounded susceptible trees separately.

A positive correlation was found between the width of the bark and the size of the inner resin canals. However, for outer resin canal size, this positive correlation was only found at the second and third sampling periods (Table 3.9).

Table 3.9. Correlation coefficients and levels of significance for correlations between bark thickness and resin canal size at four times during the growing season.

	Mar 28/29	May 14/15	Jul 6/7	Aug 25/26
Bark Thickness vs. Inner Canal Size	0.50 $p<0.01$	0.44 $p<0.01$	0.57 $p<0.01$	0.60 $p<0.01$
Bark Thickness vs. Outer Canal Size	-0.03 $p=0.87$	0.57 $p<0.01$	0.28 $p=0.09$	0.21 $p=0.20$

3.3.5 Xylem Growth

The width of the second growth ring of the leader (grown in the season when experiments were conducted) was not found to differ significantly between trees from resistant and susceptible families ($F_{(1,85)}=0.00$, $p=0.99$) or

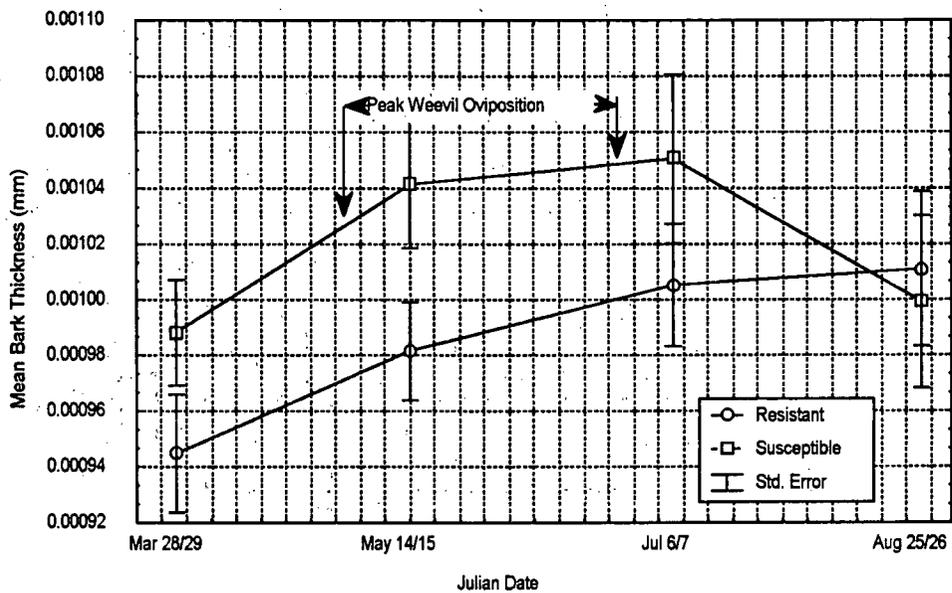


Figure 3.14. Mean bark thickness of weevil resistant and susceptible Sitka spruce trees at four times during the growing season, pooled over both sites and including both wounded and unwounded trees.

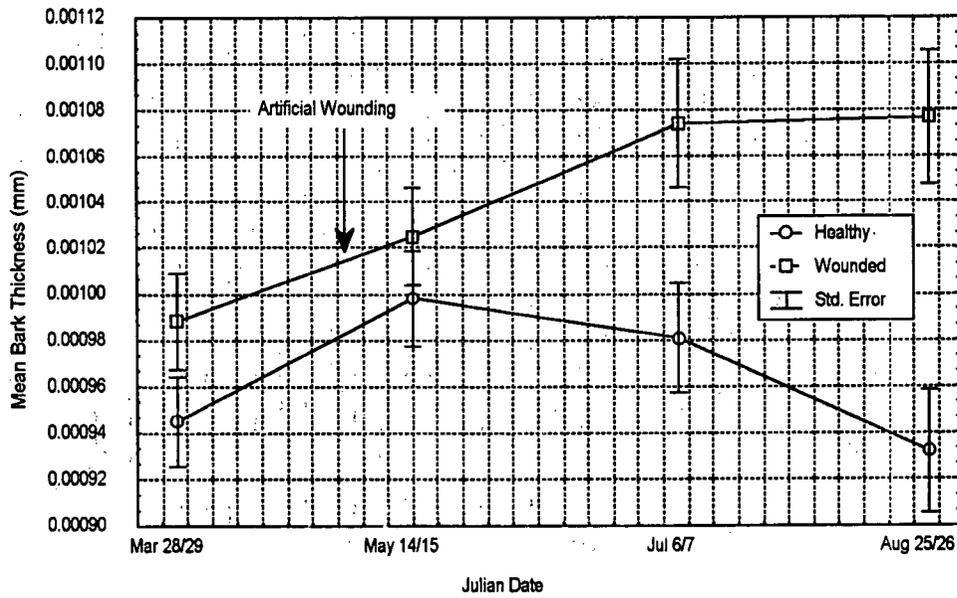


Figure 3.15. Mean bark thickness of wounded and unwounded Sitka spruce trees at four times during the growing season, pooled over both sites and weevil resistance categories.

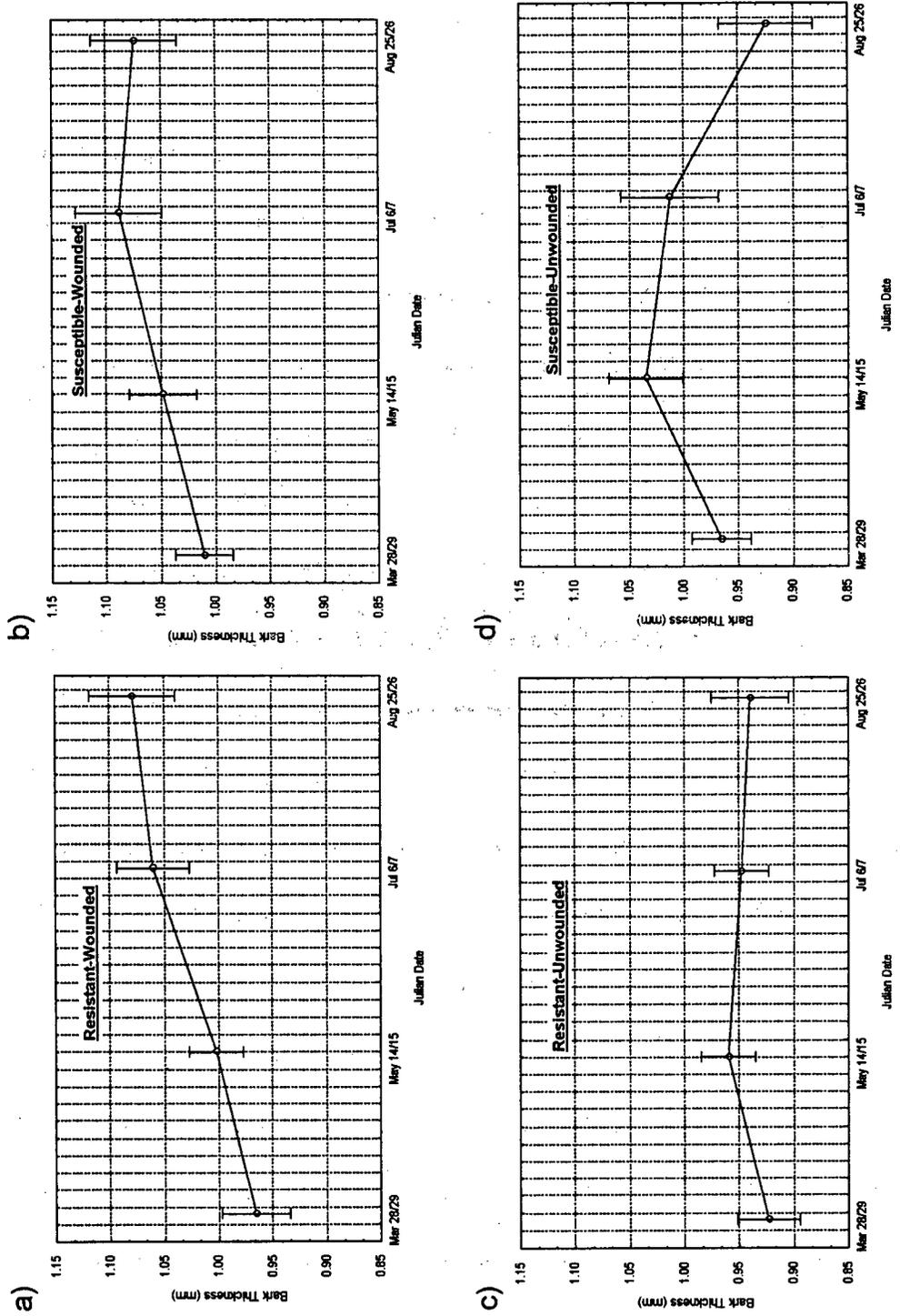


Figure 3.16. Mean bark thickness of trees at four times during the growing season, pooled over both sites for: a) wounded resistant trees, b) wounded susceptible trees, c)unwounded resistant trees, and d)unwounded susceptible trees.

between wounded and unwounded trees ($F_{(1,85)}=2.92$, $p=0.09$). In order to account for differences in prior growth rate in the above analyses, the width of the first growth ring was used as a covariate.

3.3.6 Traumatic Resin Response

In an analysis of wounded trees only, no differences were found in the traumatic response of trees from resistant and susceptible families at the first three sampling dates. No response was present at the first sampling date because wounding had not yet occurred. However, by the fourth date (August 26,27), the trees from resistant families had a significantly higher traumatic response than the susceptible ones ($F_{(1,43)}=4.48$, $p=0.04$) (Figure 3.17). When analyzing the Jordan River site alone, the traumatic response of trees from resistant families was higher than trees from susceptible families at the 0.05 level of significance for the third measurement time ($F_{(1,21)}=4.50$) (Figure 3.18), and at the 0.066 level of significance over the third and fourth sampling periods ($F_{(1,18)}=3.82$). At Cowichan Lake, the traumatic resin response did not differ between trees from resistant and susceptible families at any time. No correlation was found between the intensity of the traumatic response and any measured characteristic of the cortical resin canal system during the second or third measurement period. During the fourth sampling period, traumatic response was positively correlated with the percentage of bark occupied by inner resin canals, inner resin canal size, and bark width; but was negatively correlated with number of inner canals/ μm^2 of bark (Table 3.10).

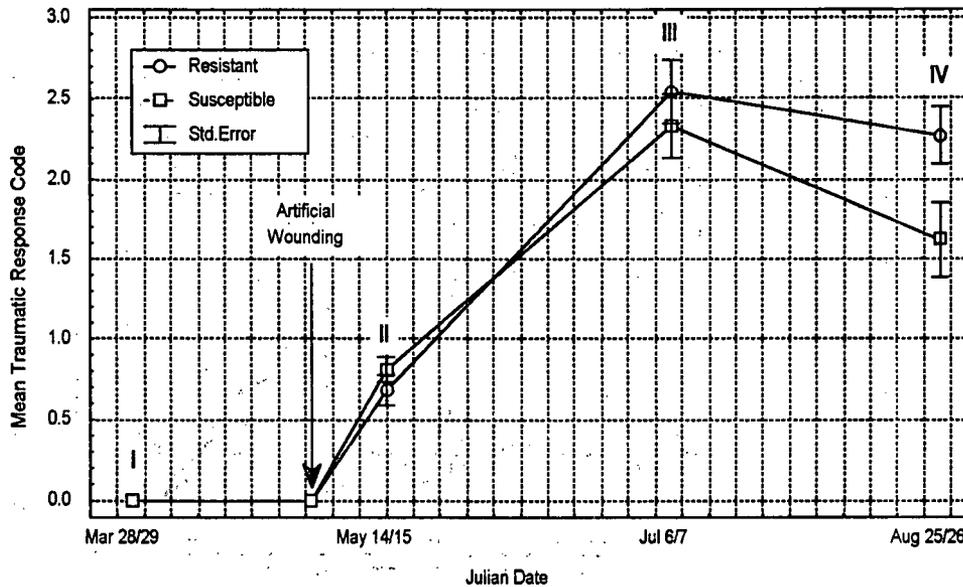


Figure 3.17. Mean traumatic response of wounded trees from weevil resistant and susceptible families at four times during the growing season, pooled over both sites. Roman numerals indicate sampling periods. Wounding was conducted between sampling period I & II. Response codes are explained in Figures 3.3a to 3.3e.

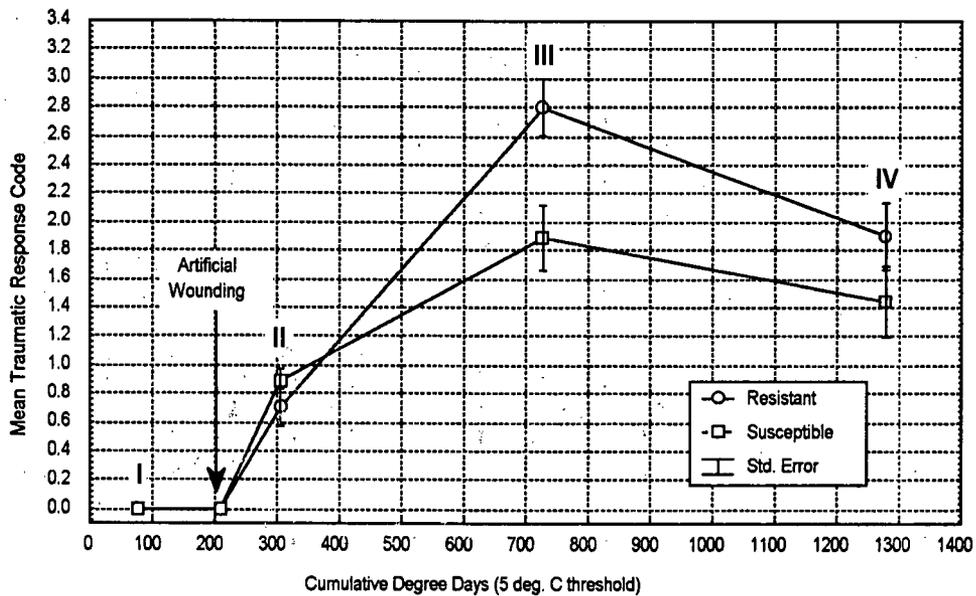


Figure 3.18. Mean traumatic response of wounded trees from weevil resistant and susceptible families at Jordan River at four times during the growing season. Roman numerals indicate sampling periods. Wounding was conducted between sampling period I & II. Response codes are explained in Figures 3.3a to 3.3e.

Table 3.10: Correlation coefficients and levels of significance for correlations between traumatic response code and constitutive resin canal system characteristics at three times during the growing season (No traumatic response occurred on March 28/29 which was prior to wounding). Correlation coefficients significant at the 0.05 level of probability are indicated by *.

Traumatic response code vs.	May 14/15	Jul 6/7	Aug 25/26
Second Growth	0.08	-0.05	0.10
Ring Width	p=0.62	p=0.75	p=0.53
% Bark Occupied by Inner Canals	-0.11 p=0.47	0.18 p=0.26	0.31 p=0.04*
% Bark Occupied by Outer Canals	0.05 p=0.74	-0.04 p=0.80	0.06 p=0.72
No. Inner Canals/ μm^2 of Bark	-0.09 p=0.56	-0.04 p=0.78	-0.33 p=0.03*
No. Outer Canals/ μm^2 of Bark	0.18 p=0.25	-0.13 p=0.41	-0.10 p=0.53
Inner Canal Size	-0.17 p=0.29	0.17 p=0.28	0.42 p=0.01*
Outer Canal Size	-0.18 p=0.44	-0.06 p=0.81	0.08 p=0.72
Bark Width	0.12 p=0.45	0.15 p=0.33	0.32 p=0.04*

3.4 DISCUSSION

A summary of factors associated with the resistance of spruce to the white pine weevil in the families studied is presented in Table 3.11.

Table 3.11. Summary of factors associated with resistance or susceptibility of Sitka spruce to the white pine weevil as revealed in this study. * Indicates resistant trees exhibited the trait to a significantly greater degree than susceptible trees ($p < 0.05$).

Mechanism	Type of Mechanism	Significance of Mechanism at:	
		Jordan River	Cowichan Lake
Density of Inner Resin Canals.....	Constitutive	-	-
Density of Outer Resin Canals.....	Constitutive	-	-
% Bark Occupied by Inner Resin Canals.....	Constitutive	*	-
% Bark Occupied by Outer Resin Canals.....	Constitutive	-	-
Size of Inner Resin Canals....	Constitutive	*	-
Size of Outer Resin Canals....	Constitutive	*	-
Increase in Size of Inner Resin Canals.....	Induced	*	-
Increase in Size of Outer Resin Canals.....	Induced	*	*
Bark Thickness.....	Constitutive	*	*
Traumatic Resin Response....	Induced	*	-

Strong evidence was found that the percentage of bark occupied by inner resin canals was associated with resistance in the Jordan River trial (Figure 3.6, Table 3.2). This confirms the findings of Alfaro (1996a), and indicates that percentage of bark occupied by resin canals is likely a more important component of resistance than the number of resin canals. The reason for this is likely because the relative area measurement accounts for the size of the resin canals as well as for the number. The size of inner resin canals and the

percentage of bark occupied by inner canals was positively correlated, and trees from resistant families were found to have significantly larger inner resin ducts than those from susceptible families (Figure 3.10, Table 3.5).

Although the trees from resistant families appeared to have larger outer resin canals than susceptible ones, this difference occurred primarily during the latter two measurement dates which were beyond the main oviposition period of the weevil. These larger canals may affect the success of larval feeding and pupation later in the year, but there was no apparent difference between trees from resistant and susceptible families in the percentage of bark occupied by outer resin canals. This suggests that, structurally, outer ducts may be of lesser importance in resistance to the spruce weevil than inner ducts. Although this is contrary to the findings of Tomlin and Borden (1997a), the familial differences in the material used by Tomlin and Borden and this study may account for the difference.

The hypothesis of a dilution effect on the number of resin canals through the growing season presented by Alfaro (1996a) is supported by this study; however, no significant difference was found between trees from resistant and susceptible families in the rate of dilution. The number of inner resin canals per unit area of bark was significantly higher in resistant trees than susceptible trees only at the start of the season (Mar 28/29). The number of inner and outer resin canals per square micron of bark decreased at a similar rate between trees from resistant and susceptible families, suggesting that the constitutive resin canal resistance of a given internode may decrease over time. Although resistant and

susceptible trees differed in their initial density of inner resin canals, there is no indication that the number of resin canals per unit of bark area is associated with resistance to the weevil in these families. The effect of a decrease in the number of resin canals could be partly offset by the increase in resin canal size over time. Tomlin and Borden (1997a) found that resistant Sitka spruce provenances and families from a wide range of locations had a significantly higher number of resin canals on average. A notable exception was the Big Qualicum provenance, to which all families in this study belong, except family 41. Tomlin and Borden (1997a) hypothesized that families from this area may rely more on other resistance mechanisms. The lack of significant differences between trees from resistant and susceptible families in this study suggests that this may be the case with other provenances as well as Big Qualicum.

A lack of any difference in the number of inner or outer resin canals per square micron of bark between wounded and unwounded trees is consistent with the fact that cortical resin canals are produced during shoot growth and that new resin canals are not produced in response to wounding (Jou 1971, Walter *et al.* 1989). An increase in both inner and outer resin duct size of trees from resistant families following wounding could be related to an increase in resin flow (Ruel *et al.* 1998) following wounding or to a reactivation of the secretory functions of the resin ducts as outlined by Walter *et al.* (1989). The reason this increase occurred to a greater extent in resistant trees than in susceptible trees is unknown; but it is possible that the reactivation of resin duct secretory functions occurs to a much lesser extent or may not occur at all in susceptible trees. The

increase in size, however, was insufficient to cause an increase in total percentage of bark occupied by resin canals.

Tomlin and Borden (1997a) suggest that a combination of thin bark and a high density of resin canals is an important trait in resistance to the spruce weevil. Trees from resistant families in this study were found to have thinner bark compared to those from susceptible families, however, no difference in number of resin canals per unit area of bark was found. This led to the conclusion that thin bark and large resin canals could also be an important resistance factor, because either this combination or the one suggested by Tomlin and Borden (1997a) would lead to a greater percentage of bark occupied by resin canals.

The fact that bark width increased following wounding seems counter intuitive given the above hypothesis. Following wounding, trees often produce wound periderms which are specialized cells that block the advance of microbial infection (Shigo 1984). Tomlin (1996) observed that lesion size increases linearly with time following mechanical wounding, so it is possible that the production of wound periderm was responsible for the observed increase in bark thickness following wounding.

In keeping with the findings of the leader measurement study done in Chapter two; measurements of the second growth ring of trees from resistant and susceptible families revealed no difference between the two at any time. Additionally, the width of the second xylem ring was not correlated with the intensity of the traumatic response at any time, suggesting that there is no short-

term trade-off between growth and resistance to the spruce weevil.

Furthermore, no evidence was found to suggest that there is a trade-off between constitutive and induced defenses, which supports the theory of Tomlin *et al.* (1998) that constitutive and induced defenses are separate resistance mechanisms. It could also be that wound at the scale done in this study does not require large scale expenditures of energy by the tree. Natural weevil woundings may have higher energy requirements.

By the second measurement date, resin soaking (Figure 3.3b) was observed in the xylem of both trees from resistant and susceptible families after wounding, but the overall response did not differ between the two. The formation of traumatic resin canals was seen extensively by the third and fourth sampling times, which is consistent with previous observations that, although other reactions may occur earlier (resin soaking), traumatic resin ducts were not formed until approximately 20 days following wounding (Wong and Berryman 1977, Kuroda and Shimaji 1983). The scale used to rank the traumatic response in this study was based only on visible changes, however it is possible that a biochemical response may occur prior to any visible response. By the third and fourth measurement dates, trees from resistant families had produced a greater traumatic response than trees from susceptible families which suggests that, like white spruce (Alfaro *et al.* 1996b), the traumatic response may also be an important mechanism of resistance in Sitka spruce against the white pine weevil.

The level of traumatic response produced by Sitka spruce in this study was much lower than was observed in white spruce by Alfaro *et al.* (1996b). The

difference could be due to several causes. When studying the traumatic response, Alfaro *et al.* (1996b) used leaders that had been attacked by weevils, whereas mechanical wounding was used in this study, in order to ensure a large sample size. Natural wounding by weevils consists of sometimes more than 100 feeding and oviposition holes and therefore, can be more intense than the mechanical wounding used here. Also, weevil wounding extends over several days, whereas mechanical wounding was applied as a one-time treatment. This, combined with the fact that mechanical wounding was found to produce a lesser traumatic response than feeding and oviposition by the weevil (Tomlin 1996), could account for the difference found between the two studies. This coincides with the hypothesis of Alfaro *et al.* (1996b) that different rings of traumatic resin canals may be produced in response to different phases of attack by the weevil, and that the greatest response is produced to oviposition holes.

Another explanation could be the mild winters in coastal British Columbia which, compared to the interior of BC, could result in Sitka spruce being exposed to weevil feeding and oviposition for a greater portion of the year than white spruce (Tomlin 1996). According to the apparency theory of plant defense, trees that are more frequently attacked would rely on constitutive defenses to a greater degree than induced ones because it would be less costly for the plant (Matson and Hain 1983, Herms and Mattson 1992).

Personal observations suggested that the Jordan River plantation provided substantially better growing conditions than the Cowichan Lake site did. The differences between the two sites in the expression of resistance

mechanisms could be a function of the resistant trees at Cowichan Lake being under more growing stress. Trees under stress would be unable to devote the same amount of resources to defense as unstressed trees would, and thus would be unable to make full use of their defensive capabilities. If resistance is modified by environment, then planting of potentially resistant trees must carefully consider environmental conditions at the planting site in order to ensure that resistance does not break down. The fact that the traits measured differentiated resistant trees from susceptible ones at Jordan River but did not at Cowichan Lake indicates that the chosen traits may not be the only traits related to the resistant or susceptible status of a tree.

The identification of several traits associated with resistance in this study does not imply that other mechanisms of resistance do not exist; rather, it is likely that a number of other traits act in concert with those identified here to provide a greater total resistance than any one trait could provide individually. This study examined only the morphology of the constitutive and traumatic resin defenses, but chemical factors are also known to be effective in resistance to *Pissodes strobi* through their action on host selection and acceptance, and on weevil development (Wilkinson 1980, Berryman 1988, Alfaro 1994, Sahota *et al.* 1994, Alfaro 1996b, Tomlin and Borden 1997b, Sahota *et al.* 1998).

4. EFFECT OF WOUNDING TIME ON THE INTENSITY OF THE TRAUMATIC RESPONSE IN SPRUCE

4.1 INTRODUCTION

The extent of insect injury suffered by a tree can be greatly affected by the developmental stage of the tree (phenology) or the seasonal timing of an insect attack (Kozlowski 1969, Fernandes 1990). Many plants undergo a relatively short, intense flushing and elongation (primary growth) phase, which is followed by a longer period in which the plant allocates resources to differentiation, defense, photoassimilation and secondary growth (Herms and Mattson 1992). Evidence suggests that an early attack, before the plant can allocate resources to defense, might have a better chance of success. Reid *et al.* (1967) observed that damage to lodgepole pine from mountain pine beetle (*Dendroctonus ponderosae* Hopkins) attack occurred more rapidly and to a greater degree when the attack was initiated early in the season compared to later in the season. Thatcher and Pickard (1967) suggest that the pattern of infestation development of southern pine beetle (*Dendroctonus frontalis* Zimmerman) is related to seasonal variation in resistance mechanisms.

Similar findings have been made for the white pine weevil as well. Hulme (1995) found that resistance to the white pine weevil could be seasonally temporary, rather than permanent; and that resistant trees were overcome when artificially subjected to an early season attack. Alfaro *et al.* (1996b) observed that successful attacks on leaders only occurred early in the growing season and that attacks on leaders later in the season were unsuccessful. Early season

aggregation on leaders is believed to optimize the weevil's chance of mating and finding suitable host material for oviposition and development (Mehary *et al.* 1994).

The amount of resin produced by a tree is known to vary within the season (Christiansen *et al.* 1987). Evidence suggests that this seasonal variability also affects a tree's traumatic response. Zangerl and Rutledge (1996) observed considerable variability in the induced defense response of wild parsnip (*Pastinaca sativa*) leaves at different times during the leaf's development. In ponderosa pine, Paine (1984) found that the size of necrotic lesions produced on ponderosa pine in response to western pine beetle attack varied throughout the year. Similarly, the traumatic response of loblolly pine was relatively slow in the spring, and much faster and greater during the summer months, suggesting that trees are more resistant to southern pine beetle attack in the summer than in the spring (Paine *et al.* 1985, Stephen and Paine 1985). This seasonal variation in traumatic resin production is also believed to occur in spruce (Alfaro 1995, Alfaro *et al.* 1999).

The objective of this study was to test the hypothesis that the intensity of the traumatic response varied if wounding occurred at different times in the tree's development.

4.2 MATERIAL AND METHODS

This study was conducted inside a shade house at the Pacific Forestry Centre in Victoria, B.C., Canada from May to July, 1999. Four families of white

spruce (families 1, 2, 73, and 127), each consisting of 3 clones and containing 5 emblings per clone were used in this study. These families originated from the full-sib crosses of parent spruce from the Prince George selection unit and Wisconsin, USA. These families were not specifically ranked for resistance to the weevil, but were assumed to be resistant based on the mean weevil resistance ranking of the parent genotypes. Emblings are vegetative propagules produced from embryos through tissue culture in the process of somatic embryogenesis (Roberts 1994). All emblings descended from a single embryo are members of a single clone.

One embling from each clone was wounded at one of five different times during the growing season. The first treatment was done on April 12, the second on April 19, the third on April 26, the fourth on May 3, and the final wounding occurred on Jun 25. Leaders were collected for processing on August 3. Only two of the four families (families 73 and 127) were wounded on the first date; the other two families (1 and 2) were added to the study subsequent to this time. Wounding was done using the same method as in Chapter 3, making eight holes on three sides of the leader. The developmental stage of the apical bud was recorded for every tree at each wounding time, using the system developed by Alfaro *et al.* (1999).

A five centimetre section was taken from the distal end of each leader, fixed in FAA, and prepared and scanned as described in Chapter 3. The parameters measured used the same methodology as section 3.2.2. and

included: number and area of resin canals (inner and outer) and bark, bark thickness, xylem ring widths, and traumatic resin response score.

4.2.1 Data Analyses

A Kruskal-Wallis non-parametric analysis of variance (ANOVA) was used to determine whether there was any difference in mean bud stage among the different families at each wounding date. This was to determine if significant bud development had occurred from one wounding date to the next. Histograms were used to examine the frequency that each bud stage was observed at the different wounding times. The traumatic resin response at the earliest wounding date was compared with the response at the other wounding dates using an ANOVA and a planned comparison. An ANOVA was also used to compare the traumatic response produced by trees at different developmental stages. The model used for the ANOVA was: $Y_{ij} = \mu + T_j + \varepsilon_{ij}$ where Y_{ij} was the traumatic response level produced, μ was the population mean, T_j was the wounding date (or developmental stage of the tree depending on the test being applied) (both fixed variables), and ε_{ij} was the experimental error.

4.3 RESULTS AND DISCUSSION

As expected, a significant increase in bud stage was observed at each consecutive wounding time ($H_{(4,54)}=45.07$, $p=0.00$) (Figure 4.1), indicating that the trees had developed significantly from one wounding time to the next. The frequency distribution of trees at a given bud stage at each wounding time

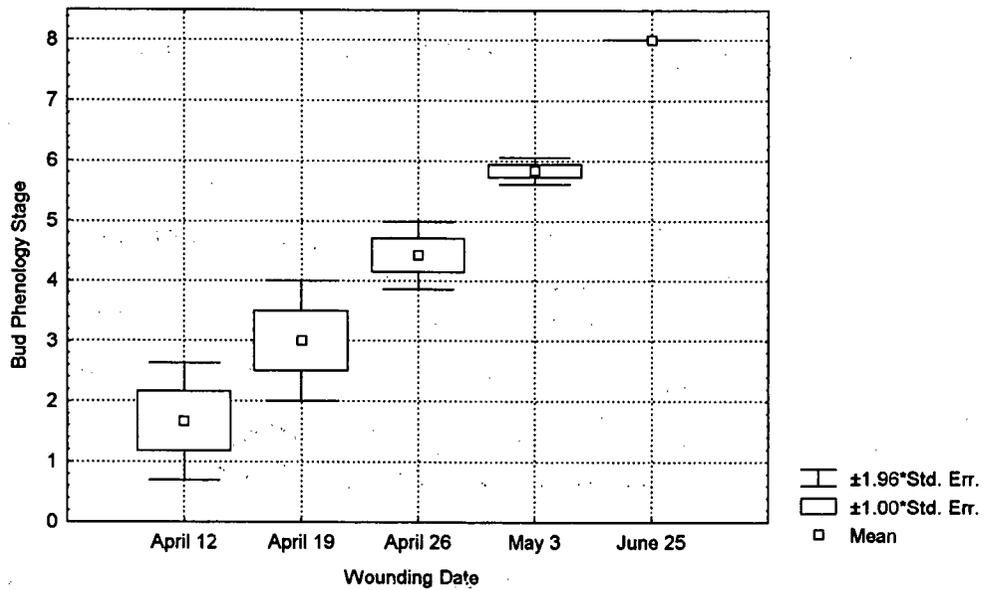


Figure 4.1. Mean apical bud stage of white spruce at five different wounding times.

supports the above finding, but demonstrates that not all developmental stages are well represented. (Figure 4.2). This can be attributed to the small sample size, which did not allow an adequate sample of all developmental stages.

No significant difference in bud stage was found among any of the families at any wounding date (Table 4.1). Families were not ranked for resistance to the weevil so no correlation between bud stage and resistance could be made here.

Table 4.1. H-values and degrees of freedom from a Kruskal-Wallis ANOVA comparing the apical bud stage of white spruce families wounded at five different times, and the mean apical bud stage by family.

	April 12	April 19	April 26	May 3	June 25
H-Value for Family Comparison	$H_{(1,6)}=0.07$ p=0.80	$H_{(3,12)}=1.49$ p=0.68	$H_{(3,12)}=2.54$ p=0.47	$H_{(3,12)}=2.20$ p=0.53	$H_{(3,12)}=0.00$ p=1.00
Family 1					
Mean Bud Stage	-	2.33	4.00	6.00	8.00
Std. Error of Mean	-	0.33	0.58	0.00	0.00
Family 2					
Mean Bud Stage	-	4.00	4.00	5.67	8.00
Std. Error of Mean	-	1.00	1.00	0.33	0.00
Family 73					
Mean Bud Stage	2.00	2.67	5.00	6.00	8.00
Std. Error of Mean	1.00	1.67	0.00	0.00	0.00
Family 127					
Mean Bud Stage	1.33	3.00	4.67	5.67	8.00
Std. Error of Mean	0.33	1.00	0.33	0.33	0.00

There was no overall difference in the traumatic resin response produced among the five different wounding times ($F_{(4,49)}=0.95$, $p=0.44$) nor was there a difference in the response produced by different families at each wounding time

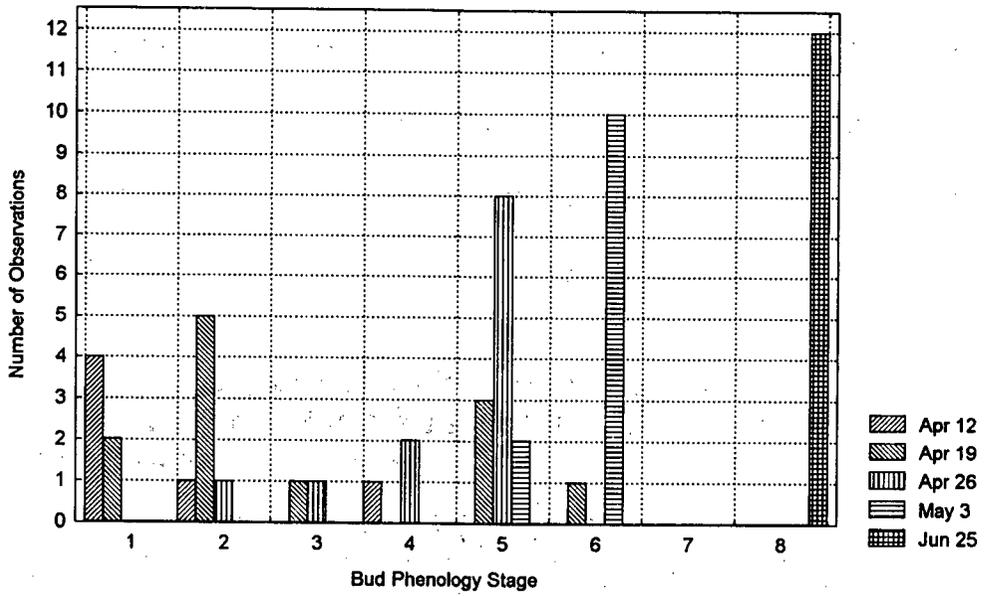


Figure 4.2. Frequency distribution of white spruce trees by bud phenology stage at five different times during the season.

(Table 4.2), but the traumatic resin response at the earliest wounding time was lower than the response at the other four wounding times (Figure 4.3). When the first wounding time was compared to the other four wounding periods using a planned comparison it was found to be different at the 0.09 level of significance ($F_{(1,49)}=2.86$). This suggests that the ability of trees to produce a traumatic resin response may be lower early in the season than later on and therefore, trees may be more susceptible to weevil attack early in the season. However, it is at this time that resin canal density is at its maximum (Chapter 3). A comparison of the traumatic response level of trees at different bud stages, however, revealed no significant difference ($F_{(6,47)}=0.36$, $p=0.90$) (Figure 4.4).

Table 4.2. F-values and degrees of freedom from an ANOVA comparing the traumatic response of families wounded at five different times, and the mean traumatic response by family.

	April 12	April 19	April 26	May 3	June 25
H-Value for Family Comparison	$F_{(1,4)}=1.00$ $p=0.37$	$F_{(3,8)}=1.33$ $p=0.33$	$F_{(3,8)}=0.44$ $p=0.73$	$F_{(3,8)}=0.41$ $p=0.75$	$F_{(3,8)}=1.93$ $p=0.20$
Family 1					
Mean Response Code	-	1.67	1.00	1.33	2.33
Std. Error of Mean	-	0.33	0.00	0.33	1.20
Family 2					
Mean Response Code	-	1.00	1.67	1.00	1.00
Std. Error of Mean	-	0.00	0.67	0.00	0.58
Family 73					
Mean Response Code	1.00	1.33	1.33	1.67	2.67
Std. Error of Mean	0.00	0.33	0.33	0.67	0.33
Family 127					
Mean Response Code	0.67	2.00	1.33	1.67	0.67
Std. Error of Mean	0.33	0.58	0.33	0.67	0.33

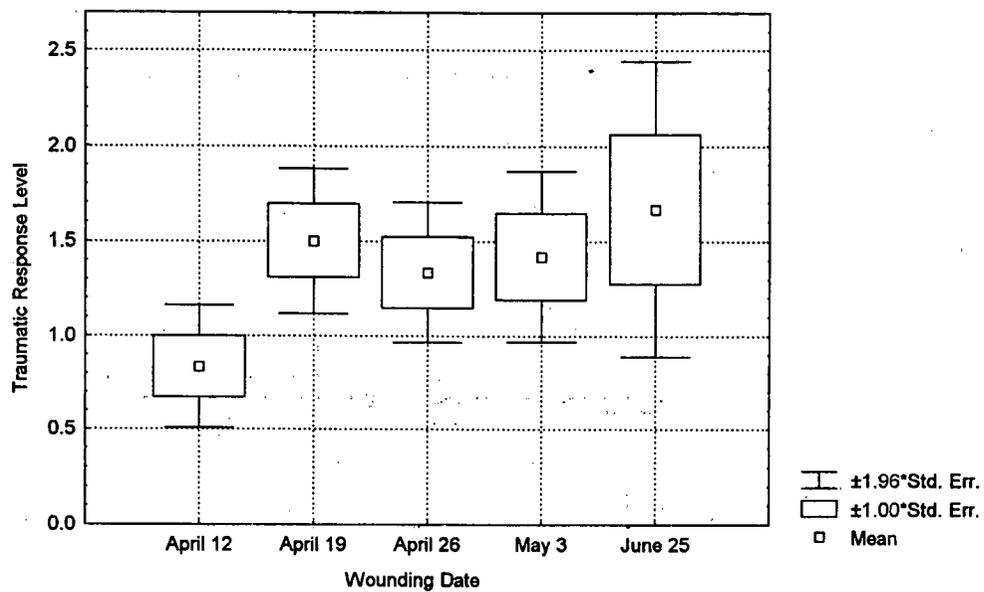


Figure 4.3. Traumatic response levels of white spruce at different times during the season.

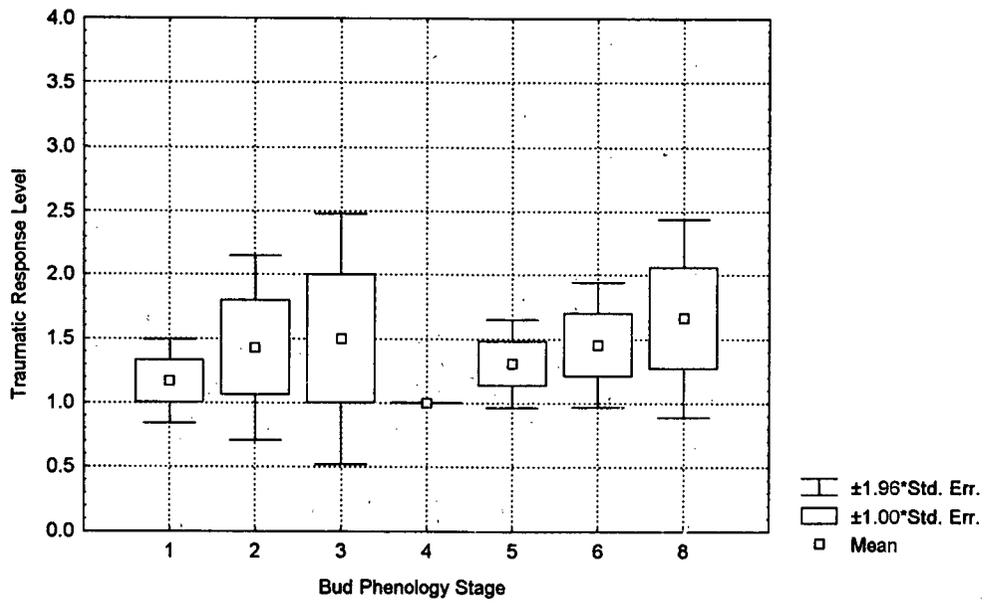


Figure 4.4. Traumatic response levels of white spruce at different stages of apical bud development.

It is possible, then that the ability to produce traumatic resin is affected to a greater degree by environmental conditions early in the season than by the tree's developmental stage. Lower temperatures are known to affect the permeability of membranes and therefore might decrease the ability of epithelial cells to secrete resin (Kramer and Kozlowski 1979). One associated wound reaction is an increase in respiration, and decreased membrane permeability results in slowed chemical reactions and therefore decreased respiration (Kramer and Kozlowski 1979). Slowed chemical reactions could also affect the ability of the tree to synthesize traumatic resin and other defensive compounds. Traumatic resin appears to be more fluid than constitutive resin (Connor 1996), and low temperatures could increase its viscosity, limiting the ability of the tree to produce a traumatic response. Taylor *et al.* (1991) observed that, in the interior of British Columbia, wetter habitats showed a higher incidence of weevil damage than drier habitats. The decreased ability to produce a traumatic response earlier in the season could also be attributed to the fact that the weather is generally wetter at this time than later in the season.

Due to the small available sample size in this study, findings should be interpreted with caution. To verify these results, future studies should be repeated using a greater number of trees, so as to ensure better representation of all bud stages. Wounding should also be carried out at a greater number of times during the season, including a treatment before trees had reached the first bud stage. This would help to confirm the finding that the traumatic response

occurs at a lower intensity early in the season.

5. THE EFFECT OF WOUND DOSAGE ON LEVEL OF TRAUMATIC RESPONSE IN SITKA SPRUCE

5.1 INTRODUCTION

In addition to timing, the success of an insect in utilizing a host plant for feeding and oviposition depends on the intensity of the attack. Bark beetles, for example, are successful in colonizing conifers if they mass-attack their host (Berryman 1972). Fernandes (1990) suggests that the defense strategy employed by a plant against a herbivore is partially dependent on the severity of the attack, which is related to the timing of the attack, the amount of tissue affected, and the type of tissue affected. Optimally, a plant should be able to respond defensively over a broad range of attack intensities, and the intensity of the defense should increase as the attack level increases. Furthermore, there must be a threshold level of attack below which it is not beneficial for the plant to initiate a defensive response, and this threshold level should decrease as the efficiency of the defense increases (Fagerstrom *et al.* 1987).

Density-dependent defense reactions have been observed in a number of different conifer species (Lunderstadt 1997). Nebeker *et al.* (1995) found that resin flow increased with the severity of wounding, and Cook and Hain (1987) suggest that the size of the lesions formed in response to bark beetle attack is partly dependent on the intensity of the attack. The activity of monoterpene cyclase, which is important in the synthesis of defensive terpenes, has also been shown to respond to wounding in a density-dependent manner (Klement and Goodman 1967, Lewinsohn *et al.* 1991).

The white pine weevil is believed to use a strategy of mass-attack through high levels of oviposition over a short time to aid in successful colonization (Alfaro 1996b). The result of this mass attack is the collapse of large portions of the resin canal system. Evidence of this strategy was found in white spruce by Alfaro *et al.* (1996b) who expected to find a linear relationship between the number of eggs laid in a leader and the traumatic response of the tree. Instead, they found a non-linear relationship, observing the maximum response in the leaders with 10-60 eggs with the response decreasing as the number of eggs per leader increased toward 250. Very few adults were found to emerge from leaders with 60 or fewer eggs, presumably because the tree was able to successfully kill the young larvae through resin soaking. Simulation of bark beetle attack has yielded similar findings. In both *Pinus* and *Picea* spp., the average resin output at the reaction zone was found to decrease once an infection dose had reached a certain level (Christiansen and Horntvedt 1983, Raffa and Berryman 1983). The threshold of successful attack is believed to vary with the resistance of the tree (Christiansen and Horntvedt 1983).

Given the limited availability of carbohydrate resources, not all energy-demanding physiological processes in trees can be met simultaneously, and as a result, trade-offs must occur in resource allocation to the processes of growth and defense. When a tree undergoes a period of growth, energy available for secondary metabolism is limited, and many of the defensive compounds such as the phenolics and terpenes, which are components of traumatic resin, are products of secondary metabolism (Herms and Mattson 1992). It seems likely

that trees which flush and develop earlier will begin the production of secondary metabolites at an earlier date. This would result in a greater availability of these products for a traumatic response.

The objective of this study is to test the hypotheses that: 1. the intensity of the traumatic response in Sitka spruce will vary with the intensity of wounding; 2. early flushing trees will produce a greater response at a given wound intensity than later flushing trees.

5.2 MATERIAL AND METHODS

This study was conducted in a shade house at the Pacific Forestry Centre in Victoria, B.C., Canada from May to July, 1999. Cuttings of eight half-sib families of Sitka spruce taken in January 1997 from the Western Forest Products Saanich seed orchard were used in this study. The families were not ranked for resistance to the weevil, however four of the families used have been identified by Alfaro (unpublished data) as being early flushing families, and four have been identified as late flushing families. A family's early or late flushing classification was based on the number of cumulative degree days required for the apical bud to reach developmental stage four (Alfaro *et al.* 1999) using a 5° C developmental threshold.

Five different treatments, including three levels of wounding and two controls, were randomly applied to the trees in each family. At the lowest level of wounding 30 holes (10 holes on 3 different sides) were drilled in the leader, 45 holes (15 holes per side) were drilled for the middle level of wounding, and

60 holes (20 holes per side) were drilled at the highest level of wounding. The first control (unwounded) sample was taken at the beginning of the study, and the second control was taken at the end, in order to observe any possible change in constitutive resin canals over the course of the study. These wounding levels were selected based on previous observations made using a wounding regime of 8 holes on each of three sides and the attempt to produce greater traumatic response at higher wound levels. The trees were wounded on May 26 using the same methodology as described in chapter 3, except for the number of holes drilled; and the leaders were collected for processing on July 22. Ten trees from each family were used in total, and each treatment was replicated twice within each family.

A five centimetre section was taken from the distal end of each leader, fixed in FAA, and prepared and scanned using the same methods as in Chapter 3. The measurements of constitutive resin canals and wound response parameters were also the same as in Chapter 3.

5.2.1 Data Analysis

Analysis of variance (ANOVA) was used to determine whether there was any difference in the intensity of the traumatic response at different wounding levels, and also to determine if the response level was different between early- and late-flushing trees. The model used for the ANOVA was: $Y_{ij} = \mu + T_j + \epsilon_{ij}$ where Y_{ij} was the traumatic response level produced, μ was the population mean, T_j was the wounding level applied to the tree (or flushing status of the tree

depending on the test being applied) (a fixed variable in both cases), and ϵ_{ij} was the experimental error. Histograms were used to examine the frequency with which a given traumatic response level was observed at a given wounding intensity. Duncan's multiple range test was used to compare which of the three wounding levels differed in intensity of traumatic response.

5.3 RESULTS

An increase in the intensity of the traumatic response was observed with an increase in wound density ($F_{(2,45)}=2.38$), however this finding was only significant at the 0.10 level (Figure 5.1). Duncan's multiple range test, however, revealed a highly significant difference ($p=0.05$) between the lowest and highest wounding levels. None of the control trees were observed to produce a traumatic response. Figure 5.2 demonstrates that high intensity traumatic responses become more frequent with increasing wound density and that low intensity responses become less frequent.

A comparison of early-flushing versus late-flushing families revealed a significantly higher traumatic response level in early-flushing trees over all wounding densities ($F_{(1,46)}=17.55$, $p=0.0001$) (Figure 5.3). When broken down into different wounding levels, early-flushing trees showed significant increase in traumatic resin response with wound density ($F_{(2,21)}=5.10$, $p=0.02$), but late-flushing trees did not ($F_{(2,21)}=0.68$, $p=0.52$) (Figure 5.4).

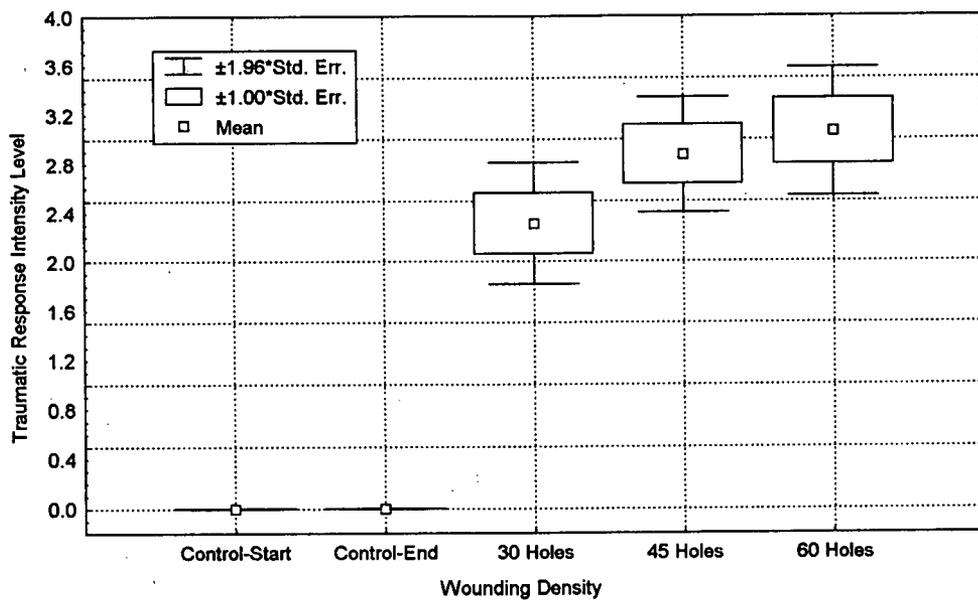


Figure 5.1. Intensity of traumatic response produced by wounding Sitka spruce clones at different levels and the level of response present in an unwounded clone collected at the beginning of the study and one collected at the end of the study.

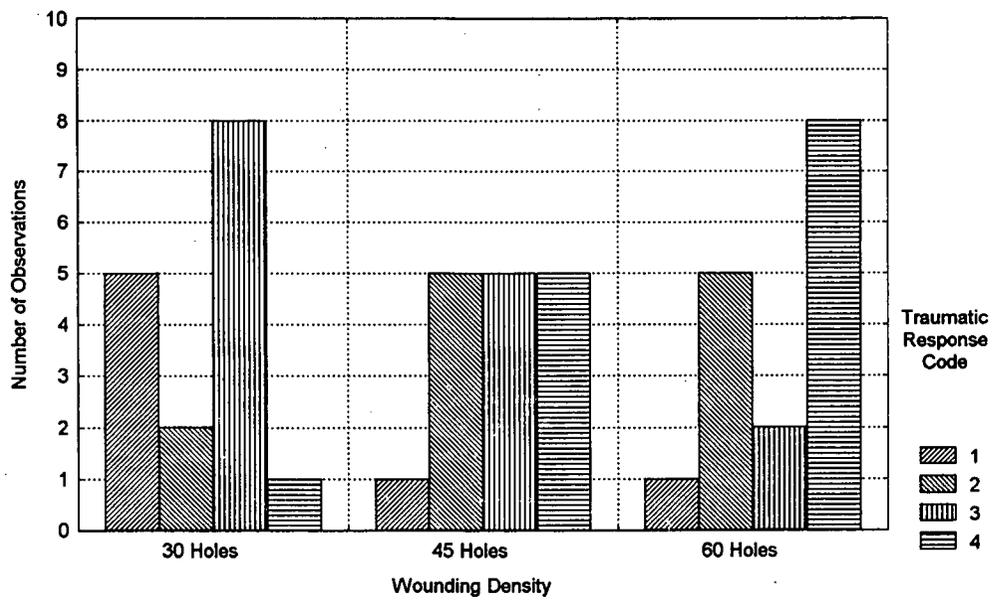


Figure 5.2. Frequency of different traumatic response levels observed at different wound densities in Sitka spruce.

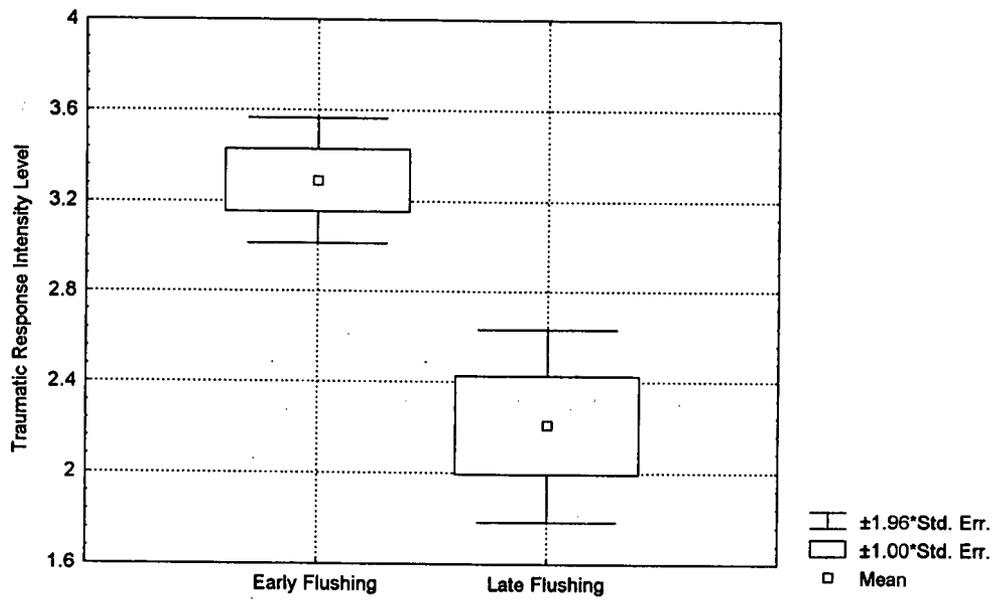


Figure 5.3. Intensity of the traumatic response of Sitka spruce produced by early and late flushing families over all wounding densities

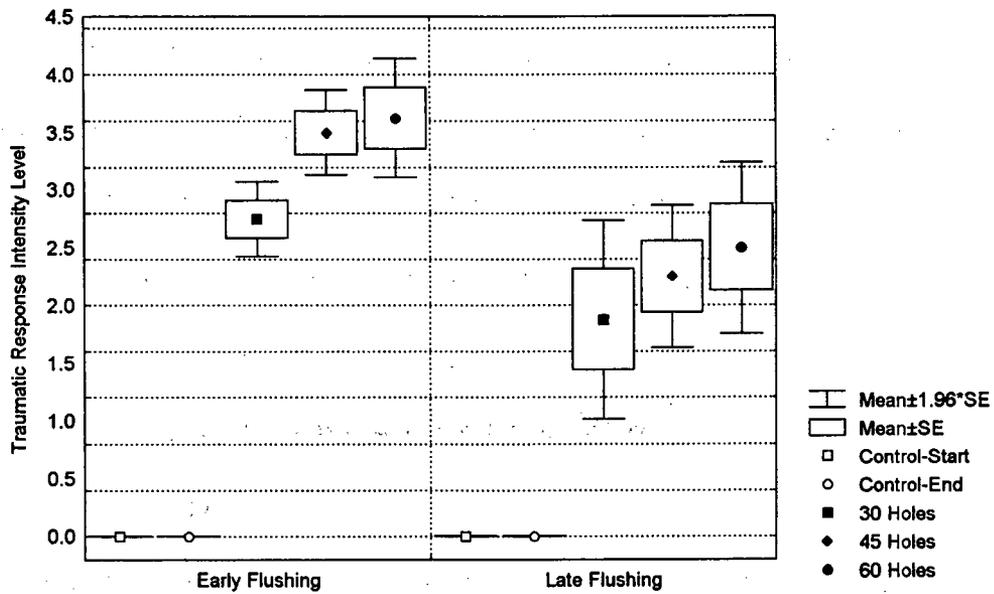


Figure 5.4. Intensity of the traumatic response produced by early and late flushing families of Sitka spruce wounded at three different densities and the level of response present in an unwounded control collected at the beginning of the study and one collected at the end of the study.

5.4 DISCUSSION

The results of this study suggest that there is a density-dependent response to the number of drill holes used to wound the leader in Sitka spruce. The fact that the two higher levels of wounding were not found to produce significantly different responses, however, suggests the existence of an upper threshold level of wounding beyond which response level is consistent. A decrease in intensity of the traumatic response at wound densities greater than those inflicted here would be consistent with the decrease in traumatic response observed by Alfaro *et al.* (1996b) at high levels of weevil-induced wounding (tissue death could occur with higher wound dosage). Given the available data, however, it is impossible to say whether this decrease in traumatic response level would occur, or whether the response level would remain stable beyond the wound levels in this study.

In a similar case, Filip *et al.* (1989) did not find any significant change in resin exudation in *Abies grandis* with increase in inoculation density of the fungus *Trichosporium symbioticum* Wright, even though a threshold for successful attack has been confirmed for *Dendroctonus ponderosae* (Hopkins) and *Ips typographicus* (L.) infestations. They suggest that their findings are based on the fact that the highest level of inoculation they used did not reach this threshold.

Because the production of a traumatic response is known to be a factor in resistance to the spruce weevil (Alfaro 1996b, Alfaro *et al.* 1996b, Chapter 3 this study), a decrease in response at wound densities greater than used in this

study would provide support for the theory that weevils must use a mass-attack strategy in order to quickly overcome the trees defenses (Alfaro 1996b, Alfaro *et al.* 1996b). This mass-attack, unlike the case of bark beetles where many insects attack the tree, involves the mass deposition of eggs over a short period. The developing larvae then behave in a mass-attack fashion.

Chapter 2 revealed that resistant trees flushed faster than susceptible ones and Chapter 3 demonstrated that the traumatic response was greater in resistant trees than susceptible ones. The importance of phenology in resistance to the spruce weevil is further emphasized by this study; and the findings of the two previous chapters are tied together by the observation that early-flushing trees are capable of producing a more intense response to wounding than later-flushing trees. Trees utilize carbohydrates for growth, respiration, and the production of defensive chemicals; and it is possible that trees which flush earlier are able to build up a large supply of carbohydrates that could be devoted to the production of secondary resin constituents. Late flushing trees would not be capable of producing the same degree of traumatic response as early flushers because they would not have the same resource pool available during the period of peak weevil oviposition. For example, Dickmann and Kozlowski (1968) found that, early in the season, the newly expanding needles of red pine, *Pinus resinosa*, could not supply enough photosynthate for their own growth due to their high energy requirements. This suggests that early flushing trees would be able to pass through this period of energy deficit before peak weevil oviposition, whereas late flushing trees may not be able to.

Breeding for early bud-break has the potential to be an important component of an integrated pest management program for *Pissodes strobi*. This does not imply, however, that wholesale planting of early flushing trees should occur throughout the province. Instead, late flushing trees should continue to be planted, in some proportion, along with the early flushing trees in order to maintain the genetic diversity necessary for the trees to adapt and evolve. Furthermore, trees which break bud early are more susceptible to frost injury than late flushing trees, so the use of early flushing trees for weevil resistance should be implemented strategically, perhaps making the greatest use of this trait in areas where weevil hazard is greatest, or where the risk of late season frost is lowest. In addition, not all early flushing trees may be resistant to weevils. Alfaro *et al.* (1999) showed that some early flushing families were susceptible to weevil.

Future studies on traumatic response intensity should involve a greater number of wound intensities, so that it can be confirmed whether or not traumatic response intensity increases with wound density and then decreases beyond a threshold level of wounding. Studies should also focus on the effect of wound density on the production of secondary resin components. In addition, the effect of tree phenology on the synthesis of secondary resin components would aid in understanding potential factors limiting the production of a traumatic response.

6. FINAL CONCLUSIONS

This study has confirmed the importance of the traumatic resin response in resistance to the spruce weevil, and has indicated that the traumatic response is wound a density-dependent. Furthermore, this study has led to the identification of several components of the constitutive resin system that have implications for resistance, and while a difference in the density (number) of resin canals was found between trees from resistant and susceptible families only early in the season, both the size of the inner resin canals and the percentage of bark occupied by inner resin canals was found to be greater in trees from resistant families. As with the inner resin canals, the outer resin canals tended to be larger in trees from resistant families than in those from susceptible families, however this difference occurred primarily in the latter half of the season. The thickness of the bark also tended to be less in trees from resistant families than in those from susceptible families, which, in combination with large resin canals would decrease the likelihood of a weevil finding a suitable area for feeding and oviposition in the leader. Although the constitutive resistance (cortical resin canal density) of a leader is known to decrease as a tree grows, trees from resistant and susceptible families were not found to differ in their rates of growth.

In general, the above traits tended to be exhibited to a greater degree in resistant trees than susceptible trees, yet no single one of these traits was emphasized in every resistant individual. It is likely, then, that a combination of traits, including, but not limited to, those identified here, are responsible for

providing resistance to a tree. Certain characteristics, such as longer leaders have been associated with resistant trees in previous studies (King *et al.* 1997), but were not observed in the present study. This supports the belief of Alfaro *et al.* (1999) that there is substantial between and within family variation in the combinations of tree traits conferring resistance to spruce trees against the white pine weevil. Hunter (1992) points out that variation among individual plants in resistance to herbivores is a prerequisite for the evolution of anti-herbivore defenses. The use of different combinations of factors by the tree would likely confound the ability of the weevil to adapt to or counter the tree's ability to resist attack. Figure 6.1 shows some of the possible defenses trees use to counter the different stages of weevil attack.

It is also clear from this study that there is a strong seasonal effect on the extent to which many traits are exhibited in both resistant and susceptible trees. This suggests that the importance of a given trait in resisting the weevil may increase or decrease through the course of the season, or that certain characteristics may be more important than others at different stages of weevil attack. This temporal variation in resistance characteristics emphasizes the importance of synchrony between host and insect in the success, or successful defense, of a weevil attack. Evidence of this was also seen in the fact that resistant trees were able to produce a greater traumatic response than susceptible trees following wounding, that trees produced the lowest level of traumatic response early in the season, and that resistant trees were earlier flushing than susceptible trees. This implies that trees which flush earlier are

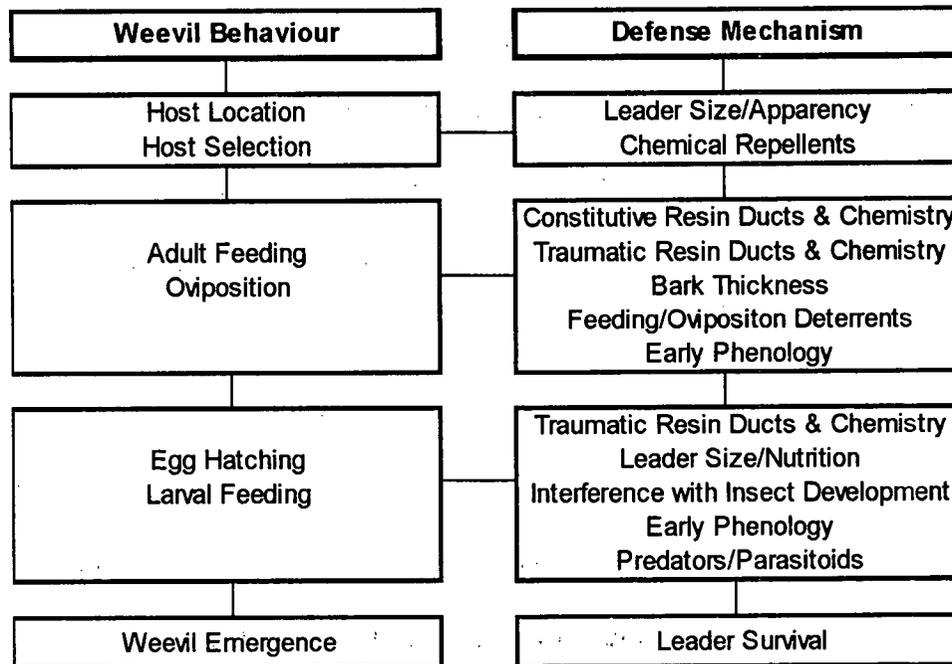


Figure 6.1. Steps in the host colonization procedure of the white pine weevil and potential host defence mechanisms used to counter each phase weevil attack.

better prepared to produce a larger traumatic response during the time of weevil attack. Similarly, Alfaro *et al.* (1999) suggests that the most resistant trees are those that are best able to maximize the efficiency of all available defense mechanisms during the period of host selection and peak oviposition.

Although early flushing in itself is not what makes a tree resistant, breeding for early budbreak would be an important component of an integrated pest management program so long as it was applied in a strategic manner which would account for other factors such as potential frost injury. Use of resistant trees must also be considered carefully in terms of environmental conditions at the proposed planting site; as there is evidence that local conditions can affect the expression of resistance. Further studies should focus on the effectiveness of certain resistance traits against different stages in the attack process. Additionally, the effects of phenology, time of year, and wound dosage on the production of attractants, repellents, terpenes, and other chemical compounds also need to be understood. Often when data are collected at the end or beginning of the season, they do not accurately represent the extent or development of defense mechanisms that an insect will face during specific stages of an attack. To understand host resistance clearly, it must be examined from the physiological time frame of the host spruces and the weevil.

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