SUSCEPTIBILITY OF SUBALPINE FIR TO WESTERN BALSAM BARK BEETLE

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Abstract

The western balsam bark beetle, *Dryocoetes confusus* Swaine, is the most destructive insect pest of subalpine fir, *Abies lasiocarpa* (Hook.) Nutt., in British Columbia (Garbutt 1992), causing scattered mortality over large areas. In order to effectively manage bark beetle populations to meet forestry objectives, it is important to understand the nature of host susceptibility.

Tree characteristics were compared between a total of 22 successfully attacked, 26 unsuccessfully attacked, and 28 control (unattacked) trees at 3 sites in the interior of British Columbia. Of 12 tree characteristics measured, five showed significant differences between successfully attacked and control trees. Successfully attacked trees had a lower percent of the bole covered with constant crown (consistent branching), lower crown volume, lower radial growth in the last 5 years, were older, and produced less induced resinosis than control trees. Larger diameter trees were also more likely to be attacked than smaller diameter trees. The relationship between recent radial growth and resistance was selected for further study.

A total of 26 fast- and 26 slow-growing subalpine fir trees were pheromone baited to induce attack by western balsam bark beetle at 2 sites in the interior of British Columbia. Although all baited trees were attacked, slow-growing trees were more likely to be successfully attacked than fast-growing trees. Fast-growing trees were more likely to produce resin, and in greater quantities, in response to attack than slow-growing trees.

A total of 20 fast- and 20 slow-growing subalpine fir trees were inoculated with a blue-stain fungus associated with western balsam bark beetle at one site in central British Columbia. Dimensions of the light and dark areas of the resultant lesions were compared between fast- and slow-growing trees and between fungus and control treatments at 3, 7, 10,

17, and 41 days after inoculation. The length and width of the dark and light areas were greater in response to fungus versus control treatments at 7, 10, 17, and 41 days after inoculation. The length of the dark and light area was significantly greater in fast-growing trees than in slow-growing trees at 7, 10, and 17 days after inoculation. There was no significant difference in the size of the lesion between fast- and slow-growing trees 41 days after inoculation.

This research shows that slow-growing trees are more susceptible to attack by western balsam bark beetle and have reduced defensive capabilities compared to fast-growing trees. These findings may explain the scattered pattern of mortality observed in stands infested with western balsam bark beetle. Western balsam bark beetle's preference for low vigour hosts and its frequent inability to induce mass attack on high vigour hosts has implications for the management of subalpine fir stands in the interior of British Columbia.

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SUSCEPTIBILITY OF SUBALPINE FIR TO WESTERN BALSAM BARK BEETLE

Chapter I. Introduction

Background and Objectives

The western balsam bark beetle, *Dryocoetes confusus* Swaine (Coleoptera: Scolytidae), is the most destructive pest of subalpine fir[1], *Abies lasiocarpa* (Hook.) Nutt., in British Columbia (Garbutt 1992). Subalpine fir is becoming an increasingly important commercial species due to depletion of mature timber at low elevation. Therefore, effective strategies and tactics for the management of western balsam bark beetle and subalpine fir are needed to ensure efficient use of the resource.

Research on western balsam bark beetle has focused primarily on developing an effective pheromone bait for the beetle (Stock 1981, 1991; Stock and Borden 1983; Borden *et al* 1987; Stock *et al* 1994a, 1994b; Camacho 1993; Camacho and Borden 1994). Pheromone baits have been used experimentally and commercially to hold and concentrate beetles in selected trees (and stands) that are subsequently harvested (Stock 1991; Stock *et al* 1994b; Harder 1998; Jeans-Williams 1999; Phero Tech Inc. undated). One project in British Columbia is currently examining stand hazard, or stand susceptibility, based on stand structure, biogeoclimatic zone, and inventory data (Maclauchlan[2], personal communication). However, little work has been done on the nature of the relationship between western balsam bark beetle and subalpine fir.

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[1] Hunt (1993) differentiates Rocky Mountain subalpine fir (*A. bifolia* A. Murray) from subalpine fir (*A. lasiocarpa*). As *A. bifolia* has not been passed to date as a new species by the International Botanical Congress and is not widely used in current literature, it will not be used in this thesis.

[2] Dr. Lorraine Maclauchlan, Entomologist, Kamloops Forest Region, BC Forest Service, Kamloops, BC.

The ability to identify trees susceptible to attack has played a key role in managing other damaging insects. However, differences in susceptibility of subalpine fir to western balsam bark beetle has not been examined at the tree level. Understanding susceptibility at the tree level would aid in the identification of high hazard stands, as well as the development of effective stand- and landscape-level management options.

For the purpose of this research, host susceptibility or resistance was defined as the characteristics or qualities of a tree that affect its likelihood of being selected for attack and/or being damaged from attack by an insect (modified from Shore and Safranyik 1992). The objective of this research was to identify and examine characteristics of subalpine fir susceptible to western balsam bark beetle. The remaining sections of Chapter I review literature pertaining to this research. Chapter II identifies parameters associated with host susceptibility, and Chapters III and IV present two experimental approaches used to test the effect of one of the identified parameters on bark beetle attack and fungus inoculation, respectively. Chapter V synthesizes the results of the three studies, suggests directions for future research, and reviews potential implications of this research on the management of western western balsam bark beetle in British Columbia.

Distribution of Western Balsam Bark Beetle and Silvical Characteristics of its Host

Western balsam bark beetle is found throughout the range of its primary host, subalpine fir. Attacks on amabilis fir (*Abies amabilis* (Dougl.) Forb.) have occasionally been reported, while Engelmann spruce (*Picea engelmanni* Parry), white spruce (*P. glauca* (Moench) Voss), and lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) are rarely attacked (Molnar 1965; Garbutt 1992).

Subalpine fir grows in high-elevation western forests. Its range extends from Alaska through the Yukon Territory and British Columbia to Oregon via the Cascade Range, and to New Mexico via the Rocky Mountains (Harlow and Harrar 1958). Although subalpine fir may be present in other ecosystems, it is most common in the Engelmann Spruce-Subalpine Fir (ESSF), Montane Spruce (MS), Spruce-Boreal Spruce (SBS), and Spruce-Willow-Birch (SWB) biogeoclimatic zones in British Columbia (Meidinger and Pojar 1991). In British Columbia, subalpine fir grows in pure or mixed stands, where it is commonly associated with Engelmann spruce, white spruce, or lodgepole pine (Meidinger and Pojar 1991). Environments dominated by subalpine fir are characterized by short growing seasons, cool summers, and very cold winters, during which soils are commonly frozen (Meidinger and Pojar 1991).

Climax spruce-subalpine fir stands in British Columbia are generally uneven-aged with both species present in the canopy and understorey (Bier *et al.* 1948). In uneven-aged stands, subalpine fir trees are often suppressed at an early age and suffer from decay, which increases with age (Bier *et al.* 1948). In contrast, subalpine fir from even-aged stands are usually less than 120 years old, rarely show signs of early suppression, have less decay, and reach saw log size by 100 years of age (Bier *et al.* 1948).

Subalpine fir has a pathological rotation age of approximately 130 years (Bier *et al.* 1948). Subalpine fir is susceptible to various root and butt rots, however, the majority of damage is caused by heart rot fungi. Red heart rot (*Stereum sanguinolentum* (Alb. and Schw.: Fr.) Fr.) and brown stringy heart rot (*Echinodontium tinctorium* (Ell. and Ev.) Ell. and Ev.) are the most common causes of decay in subalpine fir in British Columbia (Bier *et al.* 1948; Smith and Craig 1970). Susceptibility to these fungi increases with age, and

is promoted by wounding, suppression, and shade-killed branchlets (Bier *et al.* 1948; Maloy and Robinson 1968; Etheridge and Craig 1970).

Although western balsam bark beetle is the most destructive insect pest of subalpine fir, other insects may also cause damage. Where their distributions overlap in British Columbia, subalpine fir is defoliated by two-year cycle budworm (*Choristoneura biennis* Free.), eastern spruce budworm (*Choristoneura fumiferana* Clemens), and western blackheaded budworm (*Acleris gloverana* Wlshm.) (Henigman *et al.* 1999). Budworm damage usually results in growth reduction or stem defects, but successive years of defoliation may result in tree death. Defoliation may also predispose trees to attack by other insects. Attack by the balsam woolly adelgid (*Adelges piceae* Ratz.) may result in growth reduction, dead tops, and even tree death; however, it is only present in southwestern British Columbia (Henigman *et al.* 1999).

Life History of Western Balsam Bark Beetle

The western balsam bark beetle normally requires two years for the completion of its life cycle (Mathers 1931). However, Bright (1963) refers to a one-year life cycle in the western and southwestern United States, which may be due to warmer temperatures. The main flight typically commences between mid-June and mid-July when temperatures within the stand reach 15°C (Mathers 1931; Stock 1991; Hansen 1996). A second, smaller flight, which is dominated by females, occurs in August, but little flight activity takes place between flight periods (Stock 1991; Hansen 1996). The second flight may be absent or negligible at cooler sites, or during cooler summers (Hansen 1996; Gibson *et al.* 1997).

Pioneering males, responding to host volatiles, initiate attack and then produce pheromones that attract other males and females (Stock *et al.* 1983). The polygamous male excavates a nuptial chamber in the phloem or cambium where it mates on average with three to four females (Bright 1963). Mated females produce an anti-aggregation pheromone, which partially inhibits the response of both sexes to the male-produced pheromone (Stock and Borden 1983). Mated females excavate radiate egg galleries in the cambium, depositing eggs in niches along gallery walls as they progress. Parent beetles overwinter in the host. The following spring females may extend their egg galleries and lay additional eggs, before both sexes emerge and establish a third brood in a new host (Mathers 1931). Brood diapause as larvae the first winter and callow adults the second winter (Mathers 1931). Brood adults emerge the following spring or summer, thus completing the life cycle in two years.

Role of Blue-Stain Fungi Associated with Bark Beetles

Most species of bark beetles are closely associated with blue-stain fungi. Bark beetles transport spores externally on their bodies, and in specialized microbial transport structures called mycangia. Farris (1969) made the first record of mycangia in the genus *Dryocoetes*, after describing an oral pouch on each mandible of both male and female western balsam bark beetles, which contained spore-like objects. However, there was no record of the contents of the mycangia being cultured and positively identified.

The relationship between blue-stain fungi and bark beetles was originally thought to be one of mutualism (Whitney 1982); the fungi gain access to an otherwise inaccessible habitat, and in return immobilize host defenses against attacking beetles by interrupting translocation. Adults and developing brood may also benefit from the

presence of fungi through conditioning of the host substrate, as a food source (Craighead 1928; Whitney 1982), and in the production of pheromones (Brand *et al.* 1976).

Recent research has questioned the mutuality of the relationship between blue-stain fungi and bark beetles. Although many species of blue-stain fungi associated with tree-killing bark beetles are pathogenic to their respective hosts when inoculated at sufficiently high densities (Molnar 1965; Horntvedt *et al.* 1983; Christiansen and Solheim 1990, 1994; Solheim and Safranyik 1997; Krokene and Solheim 1998), the ability of blue-stain fungi to kill trees before attacking beetles effectively girdle the trees has been questioned (Parmeter *et al.* 1992; Hobson *et al.* 1994). Furthermore, active populations of southern pine beetle, *Dendroctonus frontalis* Zimm., have been discovered without their associated blue-stain fungus, *O. minus* (Hedgcock) H. and P. Sydow (Bridges *et al.* 1985). Other studies have demonstrated that while progeny of the southern pine beetle was more successful in the presence of the associated mycangial fungi complex (Barras 1973; Goldhammer *et al.* 1990), it was reduced in the presence of the *O. minus* (Barras 1970; Franklin 1970; Goldhammer *et al.* 1990). In contrast, brood of the mountain pine beetle, *Dendroctonus ponderosae* Hopk., was not inhibited by the presence of its fungal associates, *O. ips* (Rumbold) Nannf. or *O. claverigerum* (Robinson-Jeffrey and Davids.) Harrington (Nevill and Safranyik 1996). In very well developed mycangia, other species of fungi may predominate over the more virulent blue-stain fungi as well as inhibit their development in the host tree (Wingfield *et al.* 1995). Attacking beetles introduce a wide range of microorganisms into the host tree, including numerous species of non-staining fungi, yeasts, and bacteria. The role these organisms play in beetle development and their interactions with pathogenic blue-staining fungi has not been intensively studied.

The relationship between blue-stain fungi, bark beetles, and their hosts is complex and may vary between bark beetle-fungus-host systems.

Fungi Associated with Western Balsam Bark Beetle

A number of fungi have been found to be associated with western balsam bark beetle. The entomopathogen *Beauveria bassiana* (Bals.) Vuill. kills adults after they establish egg galleries (Whitney *et al.* 1984). Because *B. bassiana* does not readily colonize phloem or sapwood tissues in attacked trees, it is thought to compete poorly with other fungi that are introduced by attacking beetles (Whitney *et al.* 1984).

A number of species of blue-stain fungi have been isolated from adult western balsam bark beetles or tissues taken from attacked subalpine fir. *Ophiostoma nigrum* (Davidson) de Hoog and Scheffer, *O. minus*, and *Ceratocystis brunnea* Davidson, have been isolated from perithecia (sexual reproductive structure) fruiting in insect galleries in dead or dying subalpine fir, and may be associated with western balsam bark beetle or associated insects (Davidson 1958). *O. abiocarpum* (Davidson) Harrington, was isolated from adult beetles, as well as from perithecia in the bark of dead trees (Davidson 1958, 1966). A species of the genus *Leptographium* was commonly identified in association with blue-stain fungi in subalpine fir, but no perithecial stage was definitely connected with it (Davidson 1958). *O. dryocoetidis* (Kendrick and Molnar) de Hoog and Scheffer has been associated with adult western balsam bark beetles and isolated from necrotic tissues in unsuccessfully attacked trees (Molnar 1965). Molnar (1965) estimated that 65% of the mortality associated with western balsam bark beetle was due to *O. dryocoetidis*. Unsuccessful attacks by beetles may allow for the successful introduction of the fungi into the host. Field inoculations of *O. dryocoetidis* into subalpine fir resulted

in the formation of lesions similar to those observed in unsuccessfully attacked trees and demonstrated its pathogenicity (Molnar 1965). As a result of Molnar's work, mortality of subalpine fir attacked by western balsam bark beetle has been attributed to the beetle-fungus complex (Garbutt 1992).

Economic Impact of Western Balsam Bark Beetle in British Columbia

Western balsam bark beetle is the only species in the genus *Dryocoetes* that is capable of attacking living trees and causing significant economic losses (Bright 1963). Attacks also frequently occur on windblown and felled hosts (Stock 1991; personal observation). Western balsam bark beetle typically kills individual trees or small groups of trees that are randomly dispersed throughout the infested area (Stock 1991).

In British Columbia subalpine fir comprises approximately 13% (70 713 000 m^3) of the volume of all products billed[3] (BC Ministry of Forest 1999). Losses due to western balsam bark beetle are difficult to estimate due to the patchy nature of mortality and difficulty in interpreting the age of attack based on the colour of red needles, which may be retained for at least 5 years (Wood and Van Sickle 1989b). In beetle-infested stands, annual levels of mortality are generally less than 6% of mature subalpine fir (Stock 1991; Unger and Stewart 1992), but cumulative losses may exceed 30% of stand volume (Wood and Van Sickle 1991).

Although stand-level mortality caused by western balsam bark beetle may not be as high as other tree-killing bark beetles during epidemics, the extent and duration of the infestations result in significant mortality over time. Figure 1 shows the number of hectares infested by western balsam bark beetle annually in BC from 1985-1995. Like

[3] Volume for which stumpage has been billed. Includes waste and firmwood rejects.

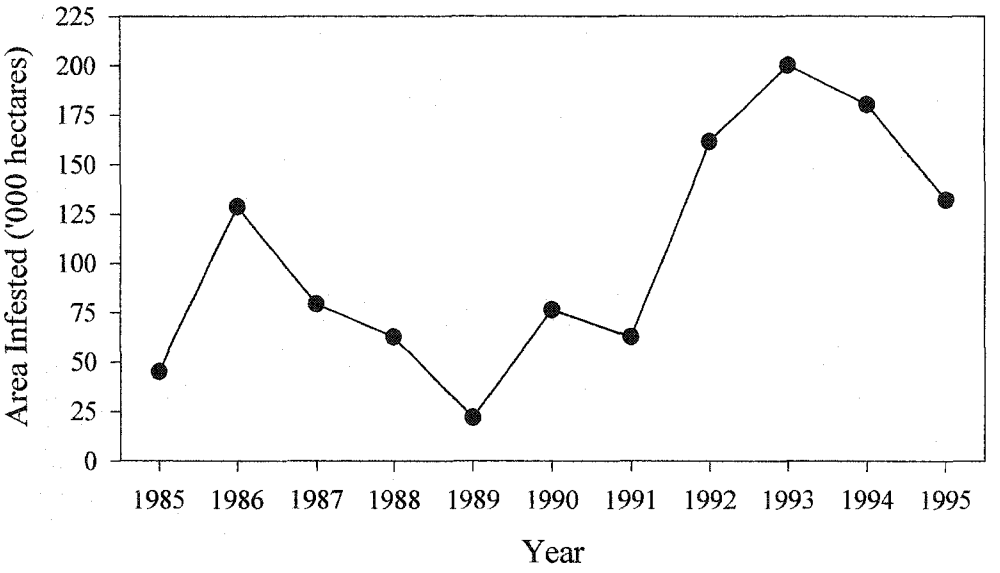

Figure 1: Area infested by western balsam bark beetle annually in BC from 1985-1995. Data compiled from Wood and Van Sickle (1986, 1987a, 1987b, 1989a, 1989b, 1990, 1991, 1993a, 1993b, 1994), and Humphreys and Van Sickle (1996).

most insects, western balsam bark beetle populations fluctuate, however, annual changes in the amount of area surveyed accounts for part of this variation (Wood and Van Sickle 1989b). Annual inconsistencies in survey coverage, and the recent inclusion of some chronically infested areas in the Prince Rupert region during aerial surveys, make it difficult to identify changes in infestation levels over time. In 1995/1996 western balsam bark beetle infested 128 000 ha province wide, compared to 165 129 ha infested by mountain pine, spruce, and Douglas-fir beetles combined (BC Ministry of Forests 1996). Seventy to 80% of the area infested by balsam bark beetle (Figure 1) usually occurs in the Prince Rupert region (Wood and Van Sickle 1986, 1987a, 1987b, 1989a, 1989b, 1990, 1991, 1993a, 1993b, 1994; Humphreys and Van Sickle 1996). Economic losses are compounded in this region, as subalpine fir comprises a quarter of its harvested timber volume (BC Ministry of Forests 1999). As other regions increase harvesting in higher

elevation areas, the economic impacts of western balsam bark beetle will spread throughout the province.

Management of Western Balsam Bark Beetle in British Columbia

Stock (1981) first provided evidence of primary and secondary attraction in western balsam bark beetle. Beetle behaviour may be manipulated at the stand level using the pheromone components *exo*-brevicomin, which has aggregative properties, or *endo*-brevicomin, which has anti-aggregative properties (Stock and Borden 1983; Stock 1991). Further analysis of beetle-produced volatiles has led to the development of more attractive baits using blends of *exo*- and *endo*-brevicomin (Camacho 1993). Field tests of the original single component bait versus the new blended bait revealed no difference in their ability to induce attack on baited trees, however, catches in pheromone-baited funnel traps were higher with the new bait (Jeans-Williams 1999). Current management tactics involve baiting blocks 1 to 2 years before harvest on a 50 m grid, using two-tree bait centers (Stock *et al.* 1994b; Harder 1998; Greenwood 1998). This approach is effective in concentrating new beetle attack around bait centers and reducing new attacks in a peripheral area for at least 50 m (Stock *et al.* 1994b; Harder 1998; Jeans-Williams 1999). The distance from which pheromone baits may effectively draw beetles has not been determined. As western balsam bark beetle infestations often cover large areas, management strategies need to be developed at the landscape level. In order to manage at the landscape level it is necessary to understand what trees and stands are most susceptible to attack. Stock (1991) identified the need to determine the factors affecting the susceptibility of subalpine fir to western balsam bark beetle 'before intelligent management objectives can be formulated'.

Chapter II. Characteristics of Subalpine Fir Susceptible to Western Balsam Bark Beetle

Introduction

Most species of bark beetles inhabit downed or dying trees (Rudinsky 1962). At high population levels, some species have the ability to mass attack and kill healthy hosts (Furniss and Carolin 1977). The specific events that trigger insect outbreaks are the subject of numerous studies, but are not well understood for most species. Epidemics of many tree-killing bark beetles have been linked to substantial increases in susceptible host material (Berryman 1972). Past studies exploring the nature of tree and stand susceptibility for major tree-killing bark beetles, such as mountain pine beetle (*Dendroctonus ponderosae* Hopkins) (Amman 1972; Waring and Pitman 1983, 1985; Shore and Safranyik 1992), spruce beetle (*Dendroctonus rufipennis* Kirby) (Hard *et al.* 1983; Safranyik *et al.* 1983), and Douglas-fir beetle (*Dendroctonus pseudotsugae* Hopkins) (Furniss *et al.* 1981; Shore *et al.* 1999), have contributed significantly to the development of relevant management strategies and options.

Western balsam bark beetle selectively kills small groups of subalpine fir at a relatively low, but constant, level each year in infested stands (Stock 1991; Unger and Stewart 1992). Although cumulative mortality may reach significant levels in chronically infested stands (Garbutt and Stewart 1991), western balsam bark beetle is less aggressive than other tree-killing bark beetles at epidemic levels. The selective and patchy distribution of mortality suggests that western bark balsam beetle may be limited by the abundance and distribution of susceptible hosts. Identifying the characteristics of

susceptible hosts may contribute to a broader understanding of the ecology of the beetle and aid in developing effective management practices.

Trees in the genus *Abies* lack extensive vertical resin canals (Bannan 1936) and rely on induced resinosis to defend against attack by bark beetles and fungi. Host vigour has been identified as the main factor affecting the ability of a tree to defend itself (Berryman 1982a). Tree vigour, usually indicated by measures of radial growth, may be influenced by senescence, defoliation, pathogens, as well as other factors that cause stress (Coulson 1979; Kaufman and Ryan 1986; Waring 1987; Yoder *et al.* 1994; Nebeker *et al.* 1995).

The objective of this study was to identify characteristics of subalpine fir trees susceptible to western balsam bark beetle.

Methods

Study Sites

Field sampling was conducted at 3 sites in the ESSF biogeoclimatic zone of British Columbia during the summers of 1998 and 1999. The Cherry Ridge (UTM 393300 5573300) and Lumby (UTM 362300 5551700) sites were located in the Thompson-Shuswap Highlands in the Vernon Forest District. The Cherry Ridge site was in the wet cold (wc2) subzone of the ESSF, where growing season moisture deficits are rare due to late snow melt and frequent summer storms (Lloyd *et al.* 1990). The elevation at this site was 1 650 m with a slope of less than 10%. The Lumby site was in the very dry cold (xc) subzone of the ESSF, which receives approximately half the annual precipitation of the wc2 (Lloyd *et al.* 1990). The elevation at this site was 1 700 m and the slope was less than 10%. The Milk River site (UTM 5914800 655000) was located in

the Rocky Mountain Trench in the Robson Valley Forest District. This site was in the transition zone between the moist mild (mm) subzone of the ESSF and the moist cold (mc) subzone of the interior cedar-hemlock (ICH) zone (Meidinger *et al.* 1988). The elevation was 1 050 m and the overall slope was less than 10%. Mature subalpine fir was the dominant species at all sites.

Survey of 1998 Attacks

In August and September 1998, a number of transects 10 m wide and 50 m apart were established at each site. All subalpine fir attacked in 1998 occurring in the transects were classified as either unsuccessful or successful attacks. Unsuccessful attacks were characterized by resin streaming on the bole, and very little or no frass present. Successful attacks were characterized by the accumulation of frass at the base, and moderate, light or no resin streaming on the bole.

Control trees were selected for half of the 1998 attacked (unsuccessful and successful) trees by identifying the nearest live, unattacked subalpine fir of similar diameter (±10%). All attacked and control trees were numbered, and their location and dbh (diameter at breast height, or 1.3 m) recorded. Any attacked trees with new resin present on the bole, were given a resin rating of light, moderate, or heavy (Table 1).

Table 1: Description of characteristics used for rating of resin streaming.

Resin Rating	Description of Resin Streaming
Light Resin	Light streaming or beads present, but a large part of bole lacks resin.
Moderate Resin	Streaming pronounced. Resin may only be present on a short length of the bole, but covers circumference at that height.
Heavy Resin	Very thick streaming. Bark was obscured by resin in the area of the bole with streaming.

Selection of Trees for Falling and Intensive Sampling

In 1998 a subset of the attacked trees and their respective controls were selected for falling and intensive sampling at each site. To ensure attacked trees selected for sampling were not attacked as a result of a spillover effect from adjacent trees, all attacked trees selected for falling were located a minimum distance of 15 m apart, with at least 2 unattacked mature subalpine fir trees between them. After falling, trees were reclassified as unsuccessful or successful attacks based on the following criteria: unsuccessful attacks had no live adults present, or had a limited number of live adults struggling in restricted pitchy galleries with no live larvae present; successful attacks had live adults and larvae present. After this reclassification, the exclusion of some misclassified 1998 unsuccessful attacks (there was resin streaming on the bole, but another insect was found mining the phloem), and the exclusion of some misclassified 1998 successful attacks (a few were determined to be 1997 successful attacks that were just starting to fade), sample sizes were as follows: 7 unsuccessful attacks, 6 successful attacks, and 8 control trees were sampled at Cherry Ridge; 11 unsuccessful attacks, 10 successful attacks, and 12 control trees were sampled at Lumby; and 8 unsuccessful attacks, 6 successful attacks, and 8 control trees were sampled at Milk River.

Sampling of Tree Characteristics

Sample measurements on each tree included dbh (cm), height (m), mean crown width (m), mean phloem thickness (mm), height to start of live crown (m), and height to start of constant crown (m). (Because the lower branches on subalpine fir are often sparse or limited to one side of the bole, the distance to the start of consistent live branching around the bole was measured and termed 'height to start of constant crown').

The percent of bole with live crown (CRTTL) and percent of bole with constant live crown (CRCNT) were calculated using total tree height and the latter two measurements respectively. Mean crown width (AVCRWD) was calculated from two measurements taken at 90-degree angles to each other. Crown volume (CRVOL) was calculated based on a cone using the following formula: $(\pi(\text{crown width}/2)^2)(\text{tree height-height to start of constant crown})/3$. Mean phloem thickness (AVPHLM) was derived from measurements taken on the east and west aspects at 1 m, 4 m, 8 m, 12 m, and 16 m. Crown foliage was classified to one of the following conditions: (1) sparse, noticeable defoliation or a very thin looking crown; (2) medium, average looking foliage for that site; or (3) heavy, above average foliage in terms of density or lushness of needles.

Stem disks were taken at 0.5 m from each felled tree, except at the Milk River site where disks were re-cut in 1999 at 1.3 m because flared and uneven growth on some of the original disks obscured the growth pattern. Two radii per disk were selected for measurement using the method outlined by Chapman and Meyer (1949). The longest diameter, from cambium to cambium, but not necessarily crossing the pith, and the diameter perpendicular to it and dissecting the pith, were measured. The sum of the diameters was divided by four, yielding the average radius of the disk, which usually occurred at only two places on the disk. Where the average radii occurred more than twice, two well-spaced radii were selected. The following growth indices were calculated using the data averaged from the two radii: cumulative growth for the last 5 years (CUM5), cumulative growth for the last 10 years (CUM10), 5 year periodic growth rate (PGR5) (growth over the last 5 years divided by growth over the 5 years previous to that), 10 year periodic growth rate (PGR10) (growth over the last 10 years divided by

growth over the 10 years previous to that), 5 by 45 year periodic growth rate (PGR5/45) (growth over the last 5 years divided by growth over the 45 years previous to that), basal area increment in the last 5 years (BAI5), and five year growth standardized by dbh (dbh divided by growth over the last 5 years). Age and canopy age (CNAGE), at 0.5 m or 1.3 m, were recorded from one of the radii. Canopy age was taken from the time when the tree showed evidence of sustained release. In cases where there was no significant sustained increase in ring width over the radii, canopy age was the same as age. Disks were measured using Windendro software (Regent Instruments Inc., Quebec City, QC, Canada) and a Hewlett-Packard ScanJet 4c/T scanner (Hewlett-Packard Ltd., Palo Alto, CA, USA).

The presence/absence of heart rot for each tree was recorded from the sanded disks because incipient rot was often missed on rough-cut stumps in the field.

Twenty Metre Radius Plots

A tally was made of all trees greater than 12.5 cm dbh within a 20 m radius of the stump by tree species. Each tree was classed according to one of the following conditions: (1) 1998 unsuccessful attack; (2) 1998 successful attack; (3) 1997 successful attack (fader), bright or dull red attack; (4) grey attack; (5) live, unattacked subalpine fir; or (6) live, species other than subalpine fir.

Stand Diameter Survey

In 1999, a number of strip plots, 20 m by 5 m, were systematically located along the established transects at each site. Species and dbh of every tree greater than 1.3 m in height occurring in the plot were recorded. Subalpine fir trees were classified according

to one of the following conditions: (1) unattacked, (2) 1998 unsuccessful attack; (3) 1998 successful attack; or (4) pre-1998 successful attack (fader, red or grey attack).

Data Analysis

Data from the strip plots were graphed and used to determine the species composition and diameter distribution at each site. Site differences in the dbh of attacked trees were tested using a one-factor analysis of variance (ANOVA). Pearson correlation matrices were used to examine the associations among growth indices and tree characteristics. The continuous variables dbh, height, age, CNAGE, CUM5, CRTTL, CRCNT, AVCRWD, CRVOL, and AVPHLM were analyzed for differences between attack classes (unsuccessful, successful, and control) and sites using a three-factor nested ANOVA. Attack class and site were entered in the model as fixed factors, and individual trees were nested under attack class and site and entered as a random factor. Variables were visually assessed for normality using histograms. A logarithmic transformation was applied to CRVOL prior to analysis to correct for non-normality. Due to small sample sizes frequency tables were used to identify potential relationships between attack class and the following: resin production rating, presence/absence of rot, and crown foliage rating. Data from the 20 m radius plots were examined using descriptive statistics as transformations failed to normalize most of the variables. The level of significance was set at 0.05 for all statistical tests. Significant ANOVAs were followed by Bonferroni's multiple comparisons test. Data were analyzed using SYSTAT 9.0 (SPSS Inc., Chicago, IL, USA).

Results

Diameter Survey

The diameter survey measured 64 trees at Cherry Ridge, 354 trees at Lumby, and 468 at Milk River. Subalpine fir was the dominant tree species at each site, followed by hybrid interior spruce, *Picea glauca x engelmannii* Engelm. (Figure 2). Western hemlock, *Tsuga heterophylla* (Raf.) Sarg., was only present at the Milk River site.

Mean diameter (±SE) of attacked trees was significantly different between sites, with the largest trees at Cherry Ridge (44.4 cm (±3.3)), followed by Milk River (31.2 cm (±1.0)), and Lumby (22.3 cm (±1.0)) ($F_{2,103} = 39.086$, $P < 0.001$, Bonferroni MCP $P < 0.001$). The majority of attacks occurred in the top three diameter classes at Lumby and Milk River (Figure 3). (Due to a small sample size, Cherry Ridge was excluded from Figure 3). Western balsam bark beetle contributed to the mortality of over three-quarters of the subalpine fir in the top two diameter classes at Lumby, and half the subalpine fir in three of the larger diameter classes at Milk River (Figure 3).

Tree Characteristics

Following dissection of the felled trees, 2 of the 8 successful attacks were reclassified as unsuccessful attacks at Cherry Ridge, 2 of the 12 successful attacks were reclassified as unsuccessful attacks at Lumby, and 3 of the 9 successful attacks were reclassified as unsuccessful attacks at Milk River. The new classifications were used for all analyses.

Tree growth indices were well correlated with each other (Appendix 1). Of the growth indices calculated, CUM5 was selected for further analyses because of its high correlation to the other growth variables, and ease of measurement.

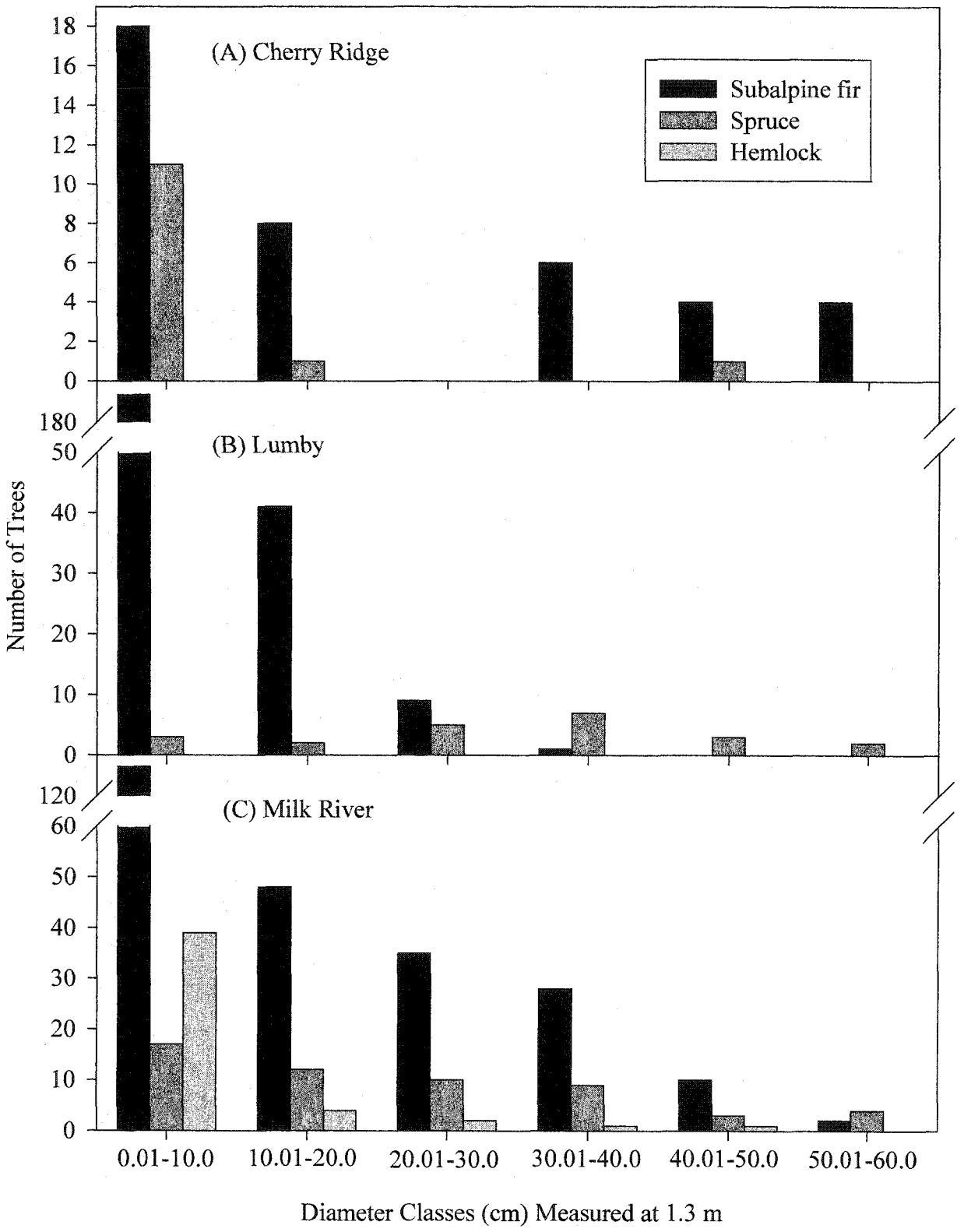

Figure 2: Diameter distribution of tree species at 3 sites.

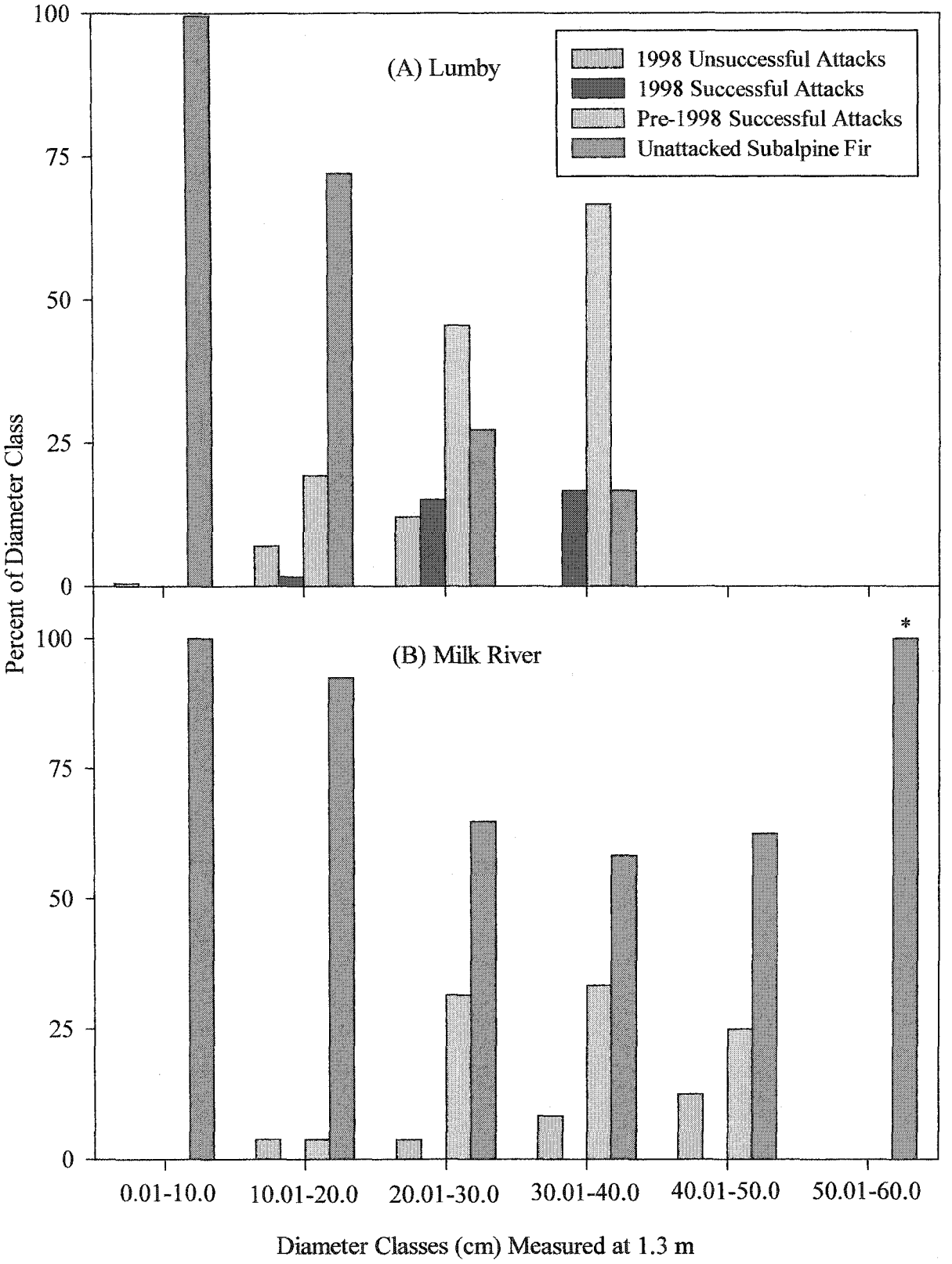

Figure 3: Standing subalpine fir by diameter class and attack status at 2 sites. Cherry Ridge has been excluded due to low sample size.
* only 2 trees were in this dbh class.

Appendix 3 lists the specific differences in tree characteristics between sites. Generally, Lumby had smaller and older trees with thinner phloem than Cherry Ridge or Milk River (Appendix 3). However, mean values of 5-year recent radial growth (CUM5) did not differ between sites (Appendix 3).

Of the tree characteristics analyzed, age, CUM5, CRCNT, and CRVOL showed significant differences between attack classes (Table 2). Successful attacks were significantly older than control trees, had significantly lower growth over the last 5 years, had a lower percent of their bole covered in constant crown, and had a smaller crown volume (Table 2). Although unsuccessful attacks did not differ significantly from successful attacks or controls in terms of age, 5-year cumulative growth, and percent of bole covered in constant crown, the means were in between those of successful attacks and controls (Table 2). Of the tree characteristics that differed significantly between attack classes, CRCNT and CRVOL were significantly correlated at all 3 sites and likely overlap in their measurement of some phenomena (Appendix 2). CUM5 and CRCNT were moderately correlated, but only at one site (Appendix 2). The remaining tree characteristics, dbh, height, CNAGE, CRTTL, AVCRWD, and AVPHLM showed no significant differences between attack classes (Table 2).

Although resin streaming occurred on the majority of attacked trees, unsuccessful attacks generally produced more resin than successful attacks (Table 3). The majority of unsuccessful attacks produced moderate quantities of resin, while the majority of successful attacks trees produced only light quantities of resin (Table 3).

The number of trees with rot present on disks appeared to vary with attack class at the Cherry Ridge and Lumby sites (Table 4). One out of 8, and 1 out of 12 control trees

had rot present at Cherry Ridge and Lumby respectively, while approximately half of the trees in the successful and unsuccessful attack classes had rot present (Table 4). At Milk River, there did not appear to be a difference in the frequency of rot between attack classes. Based on field observations of the incidence of fruiting bodies and the type of rot on the disks, the majority of rot was probably brown stringy trunk rot, *Echinodontium tinctorium* (Ell. and Ev.) Ell. and Ev. Red ring rot, *Phellinus pini* (Thore: Fr.) A. Ames, and red heart rot, *Stereum sanguinolentum* (Albertini and Schein: Fr.) Fr., were likely present to a lesser degree.

No pattern was clearly discernable between crown foliage rating and attack class (Table 5). At Cherry Ridge successful attacks appeared to have sparser crowns than unsuccessful attacks and controls. Milk River showed a similar pattern, although not as distinct, and at Lumby there was little variation in the crown foliage ratings (Table 5).

Table 2: A comparison of tree characteristics between control trees, unsuccessful attacks, and successful attacks.

	Controls Trees		Unsuccessful Attacks		Successful Attacks	
	n	Mean (SE)	*n*	Mean (SE)	*n*	Mean (SE)
Diameter*† (cm)	28	33.7 (1.9)*a*	26	34.7 (1.7)*a*	22	32.7 (1.8)*a*
Height (m)	28	24.4 (0.8)*a*	26	24.4 (0.6)*a*	22	24.2 (0.9)*a*
Age	25	179.6 (8.9)*a*	19	196.6 (12.3)*ab*	18	213.4 (12.1)*b*
Canopy Age	26	117.9 (3.7)*a*	23	116.7 (3.3)*a*	20	121.5 (2.4)*a*
5 year growth (mm)	27	4.5 (0.3)*a*	26	3.7 (0.3)*ab*	22	2.8 (0.4)*b*
% bole with live crown	28	69.0 (2.6)*a*	26	68.4 (2.4)*a*	22	62.4 (2.4)*a*
% bole with constant crown	28	56.0 (2.0)*a*	26	54.6 (1.8)*ab*	22	48.7 (1.9)*b*
Mean crown width (m)	28	3.0 (0.1)*a*	26	3.1 (0.2)*a*	22	2.8 (0.2)*a*
Crown volume (m^3)	28	36.3 (4.1)*a*	26	36.6 (4.5)*a*	22	27.0 (3.7)*b*
Mean phloem (mm)	27	5.4 (0.3)*a*	26	5.4 (0.3)*a*	22	5.7 (0.3)*a*

* Means within each variable followed by the same letter are not significantly different ANOVA, $P > 0.05$. Significant ANOVAs were followed by Bonferroni MCP, significant if $P \leq 0.05$. Crown volume was transformed to a logarithm to correct for non-normality.

† Diameter of control trees was not expected to be significantly different from unsuccessful or successful attacks because the former were selected to be of similar diameter to attacked trees.

Table 3: Number of trees in each resin category by attack class at each site.

	Cherry Ridge				Lumby				Milk River			
	No*	LR	MR	HR	No	LR	MR	HR	No	LR	MR	HR
Unsucc	0	2	4	1	0	2	7	2	0	4	4	0
Succ.	0	6	0	0	2	5	3	0	0	5	1	0

* No, no resin beads or streaming; LR, light resin; MR, moderate resin; HR, heavy resin.

Table 4: Number of trees with rot present/absent by attack class at each site.

	Cherry Ridge*		Lumby*		Milk River†	
	Rot	No Rot	Rot	No Rot	Rot	No Rot
Controls	1	7	1	11	4	4
Unsucc.	4	3	6	5	4	4
Succ.	3	3	3	7	2	4

* disks cut at 0.5 m

† disks cut at 1.3 m

Table 5: Number of trees in each crown foliage category by attack class at each site.

	Cherry Ridge			Lumby			Milk River		
	Sprs*	Mdm	Thck	Sprs	Mdm	Thck	Sprs	Mdm	Thck
Controls	1	7	0	0	9	3	0	7	1
Unsucc.	1	4	2	1	7	3	2	5	1
Succ.	3	3	0	1	8	1	3	2	1

* Sprs, sparse; Mdm, medium; and Thck, thick.

Twenty Metre Radius Plots

Figure 4 shows the mean (±SE) number of 1998 successful attacks, 1998 unsuccessful attacks, faders and red attacks, subalpine fir over 12.5 cm dbh, and other species over 12.5 cm dbh within 20 m of controls, unsuccessful attacks, and successful attacks. No clear pattern emerged between attack classes, except there were three times more successful attacks within a 20 m radius of successful attacks compared to unsuccessful attacks or control trees at Lumby (Figure 4). Density of stems over 12.5 cm dbh was higher at Lumby than Cherry Ridge or Milk River (Figure 4).

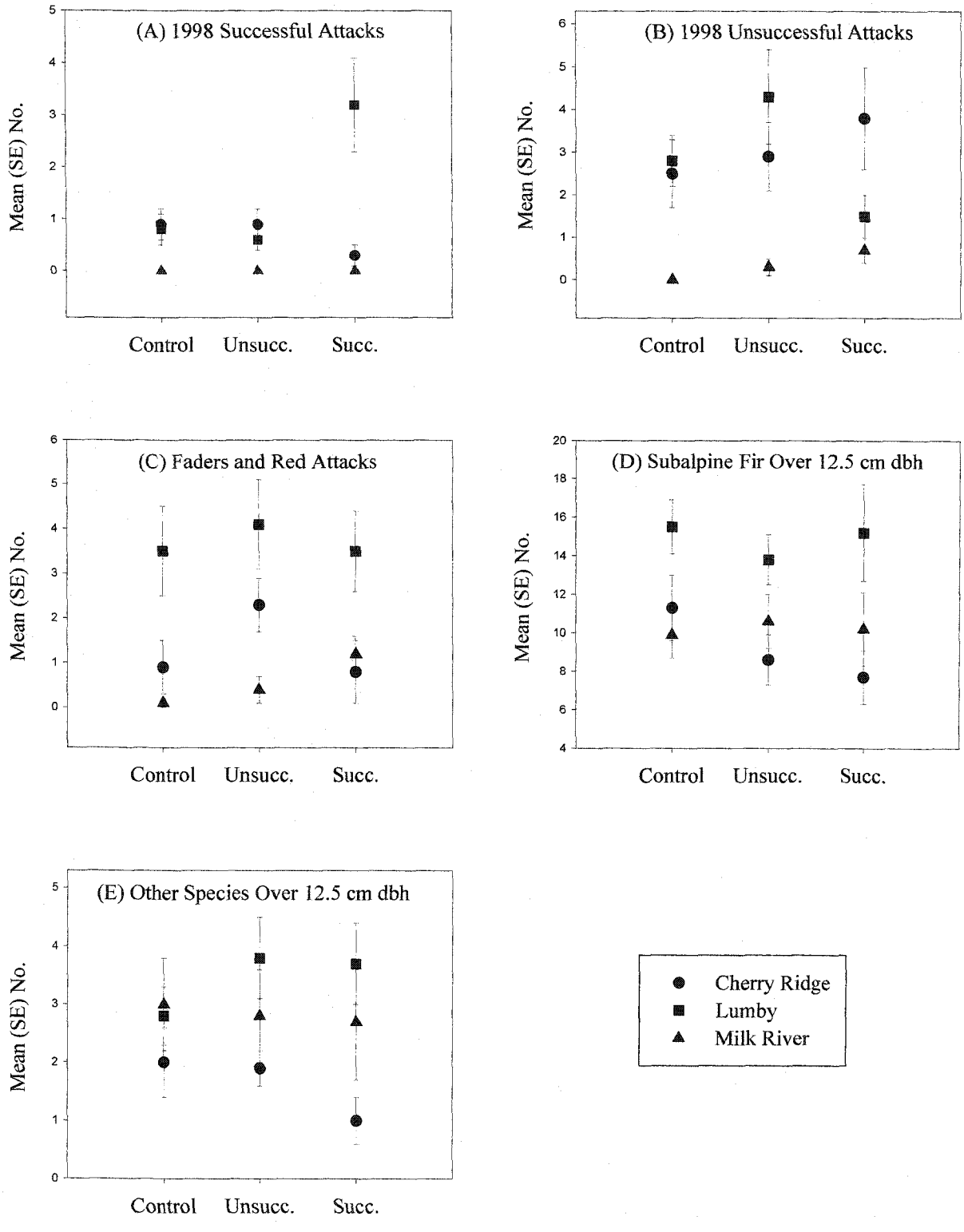

Figure 4: Mean (SE) number of (A) 1998 successful attacks, (B) 1998 unsuccessful attacks, (C) faders and red attacks, (D) subalpine fir over 12.5 cm dbh, and (E) other species over 12.5 cm dbh, occurring within a 20 m radius of control trees, unsuccessful attacks, and successful attacks at 3 sites.

Discussion

Susceptibility of subalpine fir to attack by western balsam bark beetle was associated with tree diameter, age, recent radial growth, induced resinosis, the proportion of the bole with constant crown, and crown volume.

Large diameter is a common characteristic of trees susceptible to bark beetle attack. The preference of western balsam bark beetle for larger hosts observed in this study was consistent with research on Douglas-fir, spruce, and mountain pine beetles (Cole and Amman 1969; Baker and Kemperman 1974; Furniss *et al.* 1981; Shore *et al.* 1999). Because diameter was well correlated with phloem thickness (Appendix 2; Amman 1972; Cole and Cahill 1976), larger diameter trees may provide a more suitable habitat and food source for attacking adults and developing offspring. Past studies on tree-killing bark beetles have identified phloem thickness as a major factor in determining attack, gallery, egg, and brood adult densities (Amman 1972; Cole 1973; Amman and Pace 1976; Cole and Cahill 1976; Haack *et al.* 1987b). Failure to find differences in phloem thickness between attacked and unattacked (control) trees of similar diameter suggests that attacking beetles do not differentiate between trees of the same diameter based on phloem thickness. Phloem thickness was more likely related to host suitability, not susceptibility, although it may partially explain the preference of beetles for larger diameter trees.

Western balsam bark beetle attacked trees from a wide range of diameters (9-55 cm dbh), however, small-diameter attacked trees were usually located next to larger, mass-attacked trees (personal observations). Western balsam bark beetles attracted to the larger diameter, mass-attacks may have landed on the smaller, adjacent trees as a result of increasing levels of anti-aggregation pheromones produced by mated females (Stock 1991).

Generally, the size of tree attacked depended on the diameters available in the stand. Western balsam bark beetle consistently attacked trees from the 3 to 4 largest diameter classes at each site. Thus, a large diameter, highly susceptible tree at one site, may be only a medium sized, less susceptible tree at another site. This indicates that factors other than diameter also contribute to the susceptibility of subalpine fir to western balsam bark beetle.

Tree diameter and age are usually correlated, with the oldest trees in a stand being the largest. Although *Abies* may subsist in the understorey for an extended period of time, there was a moderate correlation between diameter and age at two of the three sites (Appendix 2). Therefore, the increase in the susceptibility of subalpine fir to attack by western balsam bark beetle with tree age may be due in part to the effects of diameter. However, given that there was no significant difference in the mean diameters of unsuccessful and successful attacks (control trees were selected to be of similar diameter to attacked trees), the increase in susceptibility with age may be related to the effects of senescence. Senescence has been associated with a decline in host vigour, although the nature of the relationship has not been resolved (Coulson 1979; Kaufman and Ryan 1986; Yoder *et al.* 1994). Studies by Furniss *et al.* (1981) and Shore *et al.* (1999) have shown that Douglas-fir beetle, which prefers downed or weakened hosts, preferentially attacks older trees.

The decline in host vigour, as indicated by recent radial growth, from control trees to unsuccessful attacks to successful attacks, suggests that western balsam bark beetle may not be able to overcome the defenses of more vigourous hosts. In addition, beetles may be able to recognize low vigour hosts and preferentially select them for attack. Other studies have also associated susceptibility to bark beetle attack with host vigour. Hard (1985) found that the mean annual increment for the last 5 years was lower in both unsuccessfully and

successfully attacked spruce than unattacked spruce. Susceptibility of ponderosa pine, *Pinus ponderosa* Laws., and lodgepole pine to attack by mountain pine beetle increased with reductions in stem growth efficiency (i.e. amount of stem wood produced per unit leaf area), although beetles were less selective in areas immediately surrounding killed trees (Larsson *et al.* 1983; Waring and Pitman 1985). Reduced radial growth may increase the susceptibility of Douglas-fir to attack by Douglas-fir beetle, although study results have been contradictory. Lessard and Schmid (1990) found a higher 5-year periodic growth rate in susceptible trees, but the stands were recovering from defoliation stress. Shore *et al.* (1999) found infested Douglas-fir trees had lower growth over the last 10 years than uninfested trees (not statistically significant), but a study by Furniss *et al.* (1981) found the opposite. The differences in results may be due to combining unsuccessful and successful attacks into one category, the long time frame over which radial growth was measured, differences in site or stand parameters, or variations in bark beetle attack behaviour.

Faster-growing trees were less susceptible to attack by western balsam bark beetle than slower-growing trees, which may be due to the higher quantities of induced resinosis that were observed on fast-growing trees. Induced resinosis is responsible for repelling attacks by adult beetles, inhibiting the establishment of blue-stain fungi associated with bark beetles, deterring oviposition, and increasing brood mortality (Christiansen 1985; Berryman and Ashraf 1970; Berryman 1969; Reid *et al.* 1967). Qualitative aspects of the induced response, e.g., relative amounts of toxic compounds or viscosity, may also play an important role in defending the host against attacking organisms (Nagy *et al.* 2000; Rohde *et al.* 1996; Lewinsohn *et al.* 1993; Raffa *et al.* 1985; Raffa and Berryman 1983b; Bordasch and Berryman 1977; Wong and Berryman 1977). However, this was not examined in the current

study. It is generally accepted that host vigour affects the induced response of a host (Berryman 1982a), although the nature of the relationship, and of the induced response itself, is not fully understood.

Although subalpine fir lacks extensive primary resin canals, it has cortical resin blisters (Bannan 1936). The role of resin blisters in host defense may be limited because attacking beetles generally enter the tree in bark cracks or under flakes, thereby avoiding the blisters (personal observations). The fir engraver, *Scolytus ventralis* LeConte, was rarely observed puncturing resin blisters on grand fir, *Abies grandis* (Douglas) Lindley (Berryman 1969) or white fir, *A. concolor* (Gord. and Glend.) Lindl.ex Hildebr. (Ferrell 1983). Furthermore, resin from cortical blisters on grand fir contained only trace amounts of mildly repellent compounds to the fir engraver and its associated fungus, *Trichosporium symbioticum* Wright, whereas wound resin contained higher quantities of higher-repellency compounds (Russell and Berryman 1976; Bordasch and Berryman 1977). The location of resin blisters in the outer cortex may also limit their defensive role in larger diameter trees that have formed secondary bark (Ferrell 1983).

Pathogens, such as root diseases and stem rusts, may affect tree vigour (Oren *et al.* 1985; Lewis 1997; Mallett and Volney 1999). Past studies have demonstrated associations between trees weakened by root diseases or stem rusts, and susceptibility to bark beetle attack (Kulhavy *et al.* 1980; James and Goheen 1981; Livingston *et al.* 1983). Although roots were not excavated during this study, root diseases were likely not frequent on subalpine fir at these sites (Smith and Craig 1970). However, decay caused by heart rot fungi was common. The natural role of heart rot fungi is to contribute to the mortality of old, suppressed, or unhealthy trees in the forest (Manion 1991). Vigourous trees may be infected,

but are often able to suppress or compartmentalize decay and maintain their structural integrity. Although extensive decay in the heartwood could reduce the structural integrity of the host, the sapwood may be unaffected and remain fully functional; the effect of heart rot on tree vigour is unknown. Susceptibility of subalpine fir to heart rot increases with diameter, age, and suppressed growth (Maloy and Robinson 1968; Smith and Craig 1970; Etheridge and Craig 1976; Hunt and Etheridge 1995). Therefore trees most likely to have heart rot were also highly susceptible to western balsam bark beetle due to their physiological characteristics.

Reduced photosynthetic ability may affect the induced response, which depends on the efficient translocation of current photosynthate to the invasion site (Christiansen and Ericsson 1986; Miller and Berryman 1986). Defoliation and pruning may increase susceptibility to bark beetles or their symbiotes (Wright *et al.* 1979; Miller and Berryman 1986; Christiansen and Fjone 1993). Failure to find a relationship between crown rating and resistance of subalpine fir may have been due to the small sample size, the gross classification scheme used to rate foliage, and the lack of variation in crown ratings between trees (most were moderate). However, the tendency of successfully attacked trees to have a lower proportion of the bole covered with constant crown and smaller crown volume suggests that crown size may be related to resistance. Increased susceptibility of trees with a lower proportion of the bole with constant crown could also be due to higher rates of successful landings on such trees. Branches may inhibit incoming beetles from locating and landing on the bole. Although beetles may land lower on the bole and walk up the tree before attacking, the relatively high flight path of western balsam bark beetle as shown by Stock (1991), suggests that this beetle may also land on the upper bole.

High stand density, which may lead to reduced radial growth from competition, may be an ideal condition for the occurrence of a number of tree-killing bark beetles (Coulson *et al.* 1974; Schenk *et al.* 1977; Furniss *et al.* 1981; Shore and Safranyik 1992). However, in this study no difference was detected in the density of stems within 20 m of the sampled trees. This discrepancy could be due to differences in spatial scales, as density is usually calculated at the stand level and considered to increase susceptibility at that level. The higher number of mass attacked trees around successful attacks at Lumby may be due to secondary attraction. Dispersing beetles drawn to the mass attack by aggregation pheromones produced by male beetles may land on adjacent trees, repelled by the anti-aggregation pheromones produced by mated females (Stock and Borden 1983). Another explanation may be that susceptible hosts may occur in small groups (Stock 1991). However, this relationship was not apparent at the other two sites, or for faders and red attacks. Because of the patchy distribution of western balsam bark beetle (Stock 1991), and beetle mortality associated with flight length, a greater number of red attacks were expected in the vicinity of successful attacks. However, the beetle's preference for temporary or scattered habitats, e.g., downed or stressed hosts, may increase its inclination to disperse greater distances (Atkins 1966). The large plot radius or the rare occurrence of 1998 successful attacks, 1998 unsuccessful attacks, and faders and red attacks makes relationships hard to identify.

Conclusions

The preference of western balsam bark beetle for low vigour hosts is shared by most other species of bark beetles (Rudinsky 1962). Stressed and downed trees may emit volatiles that enable insects to locate weakened hosts. Furthermore, weakened hosts may have lowered defense systems (White 1969), which would increase the likelihood of successful

brood production. Epidemics of tree-killing bark beetles are often linked to events causing a temporary increase in susceptible host material. Assuming that events causing an increase in susceptible subalpine fir have occurred in the past, there have been few reports of western balsam bark beetle mortality levels approaching those caused by other tree-killing bark beetles during epidemics. Future research on the nature of the defense response of low and high vigour hosts may help explain western balsam bark beetle's apparent limitation to stressed or downed hosts.

Stand management practices that increase host vigour could be used to reduce mortality caused by western balsam bark beetle. The factors that affect susceptibility identified in this study should also be considered in the development of a hazard rating model for western balsam bark beetle. This study did not exhaust the list of factors that may affect host susceptibility; other important factors may include macro- and microsite characteristics, genetic variation, and the chemical composition of the induced response.

Chapter III. Success of Pheromone-Induced Attack by Western Balsam Bark Beetle and the Defense Response of Fast- and Slow-Growing Subalpine Fir

Introduction

Tree-killing bark beetles must overcome the defenses of live hosts in order to successfully colonize trees and produce brood. Plants possess two general types of defense systems to combat attacking organisms: a constitutive defense system, also known as passive, primary or preformed resistance; and an induced defense system, also known as active, secondary or induced resistance, or dynamic wound response (Klement and Goodman 1967; Berryman 1972; Merrill 1992; Paine *et al.* 1997). The constitutive defense system of a conifer consists of an extensive system of resin-filled canals that exist in the tree prior to attack. Attacking beetles may sever the preformed canals and be deterred, drowned, or physically expelled by a short-lived flow of resin. Extensive resin canals are present in many of the Pinaceae, including trees in the genera *Larix*, *Pseudotsuga, Picea*, and *Pinus* (Bannan 1936). However, trees in the genera *Abies*, *Tsuga*, and *Cedrus* lack extensive preformed resin canals, and rely mainly on the induced response for defense (Bannan 1936; Berryman 1972).

The induced defense response is a rapid reaction by a plant to mechanical wounding or invasion by organisms, e.g., insects, pathogens, viruses, or bacteria (Klement and Goodman 1967; Cates and Alexander 1982; Merrill 1992). The induced reaction involves immediate changes in the function, division, and differentiation of cells adjacent to the attack site (Rohde *et al.* 1996; Franceschi *et al.* 2000). These changes lead to cell necrosis (hypersensitivity), decreased sugar concentrations, increased monoterpene production, formation of traumatic resin ducts, induced resinosis, and wound periderm formation (Reid *et*

al. 1967; Berryman 1969, 1972; Shrimpton 1973; Wong and Berryman 1977; Raffa and Berryman 1983b, 1985; Lewisohn *et al.* 1993; Rohde *et al.* 1996; Nagy *et al.* 2000). The invading organism(s) may or may not be able to survive and overcome these obstacles. The induced defense response may be the most important factor in determining host resistance, even in trees that have a preformed defense system (Reid *et al.* 1967; Berryman 1972; Raffa and Berryman 1982a; Långström *et al.* 1992).

The ability of a tree to defend itself is often associated with host vigour, as well as the density and virulence of attacks (Berryman 1972, 1982). In Chapter II susceptibility of subalpine fir to western balsam bark beetle was related to cumulative radial growth in the last 5 years. This study further examines that relationship by using pheromone baits to induce attack on fast- and slow-growing trees. The specific objectives of this study were:

1) to compare the relative attack success of western balsam bark beetle in fast- and slow-growing trees; and
2) to compare the rate and extent of the induced defense response of fast- and slow-growing trees to attack by western balsam bark beetle.

Methods

Site Description and Tree Selection

Fifty-two trees were selected for pheromone baiting at two sites in the Engelmann spruce-subalpine fir (ESSF) biogeoclimatic zone in the interior of British Columbia. Ten fast- and 11 slow-growing trees were selected at a site (UTM 5911200 654100) in the Milk River drainage in the Robson Valley Forest District. The Milk River site was located at 1 350 m elevation on a south-facing 35% slope in the moist mild (mm) subzone of the ESSF. Sixteen fast- and 15 slow-growing trees were selected at a site (UTM 362300 5551500) south

of Lumby, in the Vernon Forest District. The Lumby site was located at 1 700 m elevation on a slope of less than 10% in the very dry cold (xc) subzone of the ESSF.

Transects 20 m wide, spaced at intervals of 50 m or 100 m at Lumby and Milk River, respectively, were established. Two cores were taken from mid to large diameter stems along the transects and used to calculate mean cumulative growth for the last 5 years (CUM5). Cores were taken perpendicular to the slope at the Milk River site, and from east and west aspects at the Lumby site, where hill slope was negligible. The mean CUM5 of control trees, unsuccessful attacks, and successful attacks identified in Chapter II (Table 2) were used to classify trees into fast- and slow-growing categories. Trees were classified as slow-growing if the mean CUM5 was 2.8 mm or less and fast-growing if the mean CUM5 was equal to or greater than 4.0 mm. Trees with a mean CUM5 between 2.8 and 4.0 mm were excluded from the study. A minimum distance of 15 m was left between fast- and slow-growing trees selected for baiting to avoid saturating the stand with pheromones.

At Milk River, selected trees ranged in age from 85 to 189 years at breast height and in diameter from 26.5 to 56.9 cm at breast height. At Lumby, selected trees ranged in age from 93 to 266 years at breast height, and in diameter from 20.8 to 37.2 cm at breast height.

Tree Baiting and Data Collection

Selected trees were baited in late May and mid June of 1999 at Milk River and Lumby respectively, prior to the main beetle flight. The standard (±)-*exo*-brevicomin bait for western balsam bark beetle (Phero Tech Inc., Delta, BC, Canada) was stapled to the north side of the bole of each tree at 2.0 m height.

Trees at the Milk River site were assessed for resin production approximately 3, 5, and 6 weeks after the main flight. At each observation time resin production was rated as

either none, light, moderate or heavy (Table 1). Trees at the Lumby site were assessed for resin production at approximately 7 weeks after the main flight.

Trees were felled approximately 6 or 7 weeks after the main flight at each site, and the following information was recorded for each tree: dbh, tree height, height of lowest and highest unsuccessful and/or successful attack if present, and attack class (Table 6). Five 400 cm^2 (20 x 20 cm) bark samples were taken at random heights and aspects (excluding the aspect that the tree lay on) in the attack zone (between the lowest and highest attack regardless of success). For each bark sample the following information was recorded if present: number of unsuccessful attacks, length and width of each lesion associated with an unsuccessful attack, number of successful attacks (gallery systems), number of female galleries in each gallery system, and the mean female gallery length per gallery system. Data from the bark samples were collated and the following variables summarizing attack were constructed for each tree:

- density of unsuccessful attacks in the attack zone;
- density of successful attacks in the attack zone;
- density of total attacks (unsuccessful and successful) in the attack zone;
- proportion of tree's total height successfully attacked;
- mean length of lesion;
- mean width of lesion;
- mean number of female galleries per gallery system; and
- mean female gallery length per gallery system.

Table 6: Description of unsuccessful, light, and successful attack classes.

Attack Status	Description of Attack
Unsuccessful	No live adults or larvae present in phloem. Lesions from unsuccessful attacks present.
Light	Live adults and larvae present. Phloem discolored around galleries, but lines of periderm present, and live phloem between attacked areas. Some lesions from unsuccessful attacks may also be present.
Successful	Live adults and larvae present. Phloem highly discolored throughout attack zone.

Data Analysis

ANOVA procedures were used to test for differences in basic tree characteristics between sites and tree growth categories. The proportions of fast- and slow-growing trees that produced resin were compared using Fisher's exact test for small sample sizes. The frequency of occurrences in tree growth categories, resin ratings, and attack classes were not statistically compared due to very small sample sizes. Variables summarizing attack were analyzed for relationships with site and tree growth using ANOVA. Many of the attack variables were not normally distributed and their distributions varied between cells. Various logarithmic, square root, and arcsine transformations failed to normalize the data because of the variety of distributions found across cells. Although a nonparametric two-factor analysis of variance exists (Zar 1999), the option of a second grouping variable was not available in a number of common statistical packages. Therefore, data from the two sites were pooled and a nonparametric one-factor ANOVA (Mann-Whitney test) was conducted for each attack variable using tree growth as the grouping variable. The tests were repeated on the attack variables using site as the grouping variable and pooling the data from fast- and slow-growing trees at each site. Results from the nonparametric tests were compared to results from parametric two-factor ANOVAs conducted on each attack variable using site and tree growth as factors. Statistical significance did not differ between the two methods and the *P*-values produced by the nonparametric tests were usually within 0.002 of the *P*-values produced by the corresponding parametric tests. Because the results of the two methods were similar, the two-factor design of the experiment, and the robustness of parametric ANOVA to violations of normality (Tabachnick and Fidell 1996), the results presented for all statistical analysis are for parametric tests performed on non-transformed data.

The level of significance was set at 0.05 for all statistical tests. Data were analyzed using SYSTAT 9.0 (SPSS Inc., Chicago, IL, USA).

Results

Site Characteristics

Mean (±SE) dbh of baited trees was significantly greater at the Milk River site than at the Lumby site (38.5 (±1.6) cm and 26.0 (±0.8) cm, respectively) ($F_{1,48} = 55.181$, $P < 0.001$). Mean dbh of fast- and slow-growing trees did not differ significantly within sites ($F_{1,48} = 0.308$, $P = 0.582$). Height of baited trees did not differ significantly between fast- and slow-growing trees ($F_{1,48} = 0.340$, $P = 0.563$) or between sites ($F_{1,48} = 1.295$, $P = 0.261$). Beetle pressure, a subjective measure based on the relative abundance of red-attacked trees observed at each site, was described as high at the Lumby site and moderate at the Milk River site.

Host Defense and Tree Growth

Beetles attacked all 52 baited trees. Six to 7 weeks after the main beetle flight, 29 of the 52 baited trees had produced resin. At the Lumby site, seven weeks after initial attack the proportions of fast- versus slow-growing trees that had produced resin were not significantly different (Fisher's exact test, $P = 0.479$) (Table 7). At Milk River, a significantly greater proportion of fast-growing trees had produced resin by seven weeks after initial attack than slow-growing trees (Fisher's exact test, $P = 0.008$) (Table 7).

Table 7: Number (percent) of fast- and slow-growing trees with resin present/absent for Lumby and Milk River research sites, 6 to 7 weeks after initial attack.

	Lumby		Milk River	
	Fast	Slow	Fast	Slow
Resin present	10 (62.5)	7 (47)	9 (90)	3 (27)
Resin absent	6 (37.5)	8 (53)	1 (10)	8 (73)

During the first observation of baited trees at the Milk River site on 21 July 1999, approximately 2-3 weeks after the main beetle flight, 2 of the 21 baited trees had resin present - 1 fast- and 1 slow-growing tree. At this time all 21 baited trees had frass present, either on the bole or accumulated at the base of the tree, indicating that all trees had received some degree of attack. On 4 August 1999, 9 of the 21 baited trees had resin present – 6 fast- and 3 slow-growing trees. By 11 August 1999, 9 of the 10 fast-growing trees had resin present, and there was no change in the number of slow-growing trees with resin present.

Fast-growing trees received slightly higher resin production ratings than slow-growing trees at the Lumby site (Table 8). This relationship was not discernable at the Milk River site due to the low number of slow-growing trees that produced resin.

The majority of slow-growing trees at both sites were successfully attacked regardless of the presence of resin or the amount of resin produced (Table 8). Fast-growing trees that produced resin were more likely to have unsuccessful or light attacks, whereas fast-growing trees that did not produce resin were more likely to have successful attacks (Table 8). Fast-growing trees that produced resin had an increased chance of resisting attack (having unsuccessful or light attacks) as resin rating increased (Table 8).

Table 8: Number and attack status of fast- and slow-growing baited trees by resin production for Lumby and Milk River research sites.

	Lumby							
	Fast-growing (n = 16)				Slow-growing (n = 15)			
	No Resin	Light Resin	Mod. Resin	Heavy Resin	No Resin	Light Resin	Mod. Resin	Heavy Resin
Unsuccessful	0	1	3	1	0	1	0	0
Light	1	2	2	0	0	1	0	0
Successful	5	1	0	0	8	5	0	0
Total	6	4	5	1	8	7	0	0

Table 8 cont'd.

	Milk River							
	Fast-growing (n = 10)				Slow-growing (n = 11)			
	No Resin	Light Resin	Mod. Resin	Heavy Resin	No Resin	Light Resin	Mod. Resin	Heavy Resin
Unsuccessful	0	2	1	0	1	0	0	0
Light	1	0	6	0	1	0	1	0
Successful	0	0	0	0	6	0	2	0
Total	1	2	7	0	8	0	3	0

Note: n values are total number of fast- or slow-growing trees at each site.

Attack Success and Tree Growth

The distribution of fast-growing trees into attack categories varied between sites (Table 9). At the Lumby site, fast-growing trees were equally divided between the 3 attack classes - unsuccessful, light, and successful (Table 9). At the Milk River site, 70% of the fast-growing trees had light attacks, while 30 % had unsuccessful attacks and no trees were successfully attacked (Table 9). The distribution of slow-growing trees into attack categories followed the same trend at both the Lumby and Milk River sites with the majority being successful attacks (86% and 73% respectively) and the remainder either light or unsuccessful attacks (Table 9).

Table 9: Number (percent) of fast- and slow-growing baited trees classified as unsuccessful, light, or successful attacks for Lumby and Milk River research sites.

	Lumby		Milk River	
	Fast	Slow	Fast	Slow
Unsuccessful attacks	5 (31)	1 (7)	3 (30)	1 (9)
Light attacks	5 (31)	1 (7)	7 (70)	2 (18)
Successful attacks	6 (38)	13 (86)	0 (0)	8 (73)

The 8 fast- and 2 slow-growing unsuccessfully attacked trees did not differ significantly in mean lesion length, mean lesion width, density of unsuccessful attacks in the

attack zone, and proportion of the bole attacked ($F_{1,6} = 0.003$, $P = 0.959$; $F_{1,6} = 0.049$, $P = 0.465$; $F_{1,6} = 0.289$, $P = 0.610$; $F_{1,6} = 105.679$, $P = 0.398$, respectively). Site had no significant effect on these variables ($F_{1,6} = 1.873$, $P = 0.220$; $F_{1,6} = 0.791$, $P = 0.408$; $F_{1,6} = 0.921$, $P = 0.374$; $F_{1,6} = 3.822$, $P = 0.098$, respectively).

The 6 fast- and 21 slow-growing successfully attacked trees did not differ significantly in density of successful attacks in the attack zone, density of total attacks in the attack zone, proportion of the bole successfully attacked, mean number of females per male, and mean gallery length per female ($F_{1,24} = 0.521$, $P = 0.477$; $F_{1,24} = 13.005$, $P = 0.001$; $F_{1,24} = 0.426$, $P = 0.520$; $F_{1,24} < 0.001$, $P = 0.996$; $F_{1,24} = 1.428$, $P = 0.244$, respectively). (Because there were no successfully attacked fast-growing trees at the Milk River site an interaction between site and tree growth was not tested for). Successfully attacked trees at Lumby had a significantly higher density of successful attacks, and total number of attacks in the attack zone than successfully attacked trees at Milk River ($F_{1,24} = 4.833$, $P = 0.038$; $F_{1,24} = 13.005$, $P = 0.001$, respectively). The mean (±SE) number of females per male for successfully attacked trees was significantly higher at Milk River (4.111 (±0.463)) than Lumby (3.243 (±0.130)), although the difference was less than one ($F_{1,24} = 5.008$, $P = 0.035$). The significantly higher mean gallery length per female in successfully attacked trees at Milk River compared to Lumby ($F_{1,24} = 5.886$, $P = 0.023$) was likely due to flight and sampling time differences.

The 12 fast- and 3 slow-growing lightly attacked trees did not differ significantly in density of successful attacks or total attacks in the attack zone, proportion of the bole successfully attacked, mean number of females per male, and mean gallery length per female ($F_{1,11} = 0.002$, $P = 0.966$; $F_{1,11} = 0.035$, $P = 0.854$; $F_{1,11} = 0.003$, $P = 0.957$; $F_{1,11} = 0.173$, $P =$

0.685; $F_{1,11} = 1.721$, $P = 0.222$, respectively). Site had no significant effect on these variables ($F_{1,11} = 0.714$, $P = 0.416$; $F_{1,11} = 0.009$, $P = 0.926$; $F_{1,11} = 0.674$, $P = 0.429$; $F_{1,11} = 0.222$, $P = 0.647$; $F_{1,11} = 0.207$, $P = 0.660$ respectively). However, lightly attacked fast-growing trees at Lumby had a higher density of successful attacks in the attack zone than lightly attacked slow-growing trees, whereas this pattern was reversed at Milk River, but the interaction was not significant ($F_{1,11} = 3.842$, $P = 0.076$).

Discussion

Resistance of subalpine fir to attack by western balsam bark beetle appeared to be associated with the quantity of resin produced. Attacking adults may be inhibited by resin production as they are physically expelled from the host, drowned in their galleries, or deterred from continuing attack by volatiles in the resin. Furthermore, induced resinosis may deter oviposition, increase brood mortality, and inhibit the establishment of blue-stain fungi associated with bark beetles (Reid *et al.* 1967; Berryman 1969; Berryman and Ashraf 1970; Shrimpton and Reid 1973; Shrimpton 1973; Raffa and Berryman 1982a, 1982b; Christiansen 1985; Hornvedt 1988). Host resistance has been linked to secondary resin production in other systems as well, e.g., lodgepole pine and mountain pine beetle (Reid *et al.* 1967; Raffa and Berryman 1982a), and grand fir and fir engraver beetle (Berryman 1969). The chemical composition of the resin may also play an important role in host defense (Kosuge 1969; Wong and Berryman 1977; Bordasch and Berryman 1977; Raffa and Berryman 1982b; Raffa *et al.* 1985; Lewinsohn *et al.* 1993; Nagy *et al.* 2000), but was not examined in this study.

Fast-growing trees were likely more resistant to attack than slow-growing trees because they were more likely to produce resin, and greater quantities of it. This was consistent with the results presented in Chapter II, which found that naturally resistant trees

were faster-growing and produced greater quantities of resin than non-resistant trees. Tree resistance has been tested with the inoculation of pathogenic blue-stain fungi and has been positively correlated with the quantity of induced resin in the wound response (Shrimpton 1973; Christiansen 1985; Hornvedt 1988; Lieutier *et al.* 1993). The relationship between host vigour, as indicated by measures of growth, and resistance to bark beetle attack has been well established (Waring and Pitman 1980, 1985; Larsson *et al.* 1983; Mitchell *et al.* 1983; Hard 1985; Shore *et al.* 1999). The results of the current study suggest that host vigour affects the quantity of resin produced, which strongly influences host resistance. However, attack success was also higher in slow-growing trees than in fast-growing trees with equivalent resin production (Table 8). This may indicate that the chemical composition of the resin or other aspects of the induced defense response, e.g., cellular necrosis or wound periderm formation, contribute to host resistance and may be less effective in slow- versus fast-growing trees. Because fast- and slow-growing trees did not appear to differ in the length of time it took to produce resin (on trees that did produce resin), this further suggests that host resistance may depend on the quantity and quality of the resin produced.

The higher density of attack on slow- versus fast-growing trees may be a function of primary attraction, secondary attraction, or a combination of both. Dispersing beetles may select a susceptible host using primary attraction through one of two scenarios. Susceptible hosts, e.g., stressed or weakened trees, may release volatiles that attract dispersing beetles to their specific location (Rudinsky 1966; Berryman 1982a). In contrast, dispersing beetles may land at random on hosts and then decide whether or not to construct galleries after experiencing the defense response of the host, or assessing the suitability of the host through initial feeding (Berryman and Ashraf 1970; Raffa and Berryman 1980). In either scenario, a

greater number of beetles could potentially select the same host independently through primary attraction. In the case of western balsam bark beetle, it is likely that the initial pioneering beetles select hosts based on primary attraction, but secondary attraction is used to guide other beetles to the selected host in order to induce a mass attack. Tree-killing bark beetles attack *en masse* in a strategy designed to overcome host resistance. As the tree responds to each attack, energy is used in the wound reaction, and the quantitative response to successive attacks is reduced (Raffa and Berryman 1982b; Miller and Berryman 1982). The 'threshold of successful attack' increases with increasing resistance of the host, and more attacks are required to overcome vigourous hosts (Waring and Pitman 1980; Berryman 1982a, 1982b). Furthermore, the shorter the duration over which attacks occur, the more likely the defenses of the host will be overwhelmed (Miller and Berryman 1982). Due to the effective defense response of fast-growing trees, pioneering beetles may be unable to establish attack quickly and release aggregation pheromones to induce a mass attack. Although the synthetic bait provides a source of attraction, western balsam bark beetle responds to increasing levels of pheromones (Stock 1991). Therefore, dispersing beetles may be drawn to the stronger pheromone plume associated with baited slow-growing trees where attack was quickly established with less resistance.

The relatively high number of fast-growing trees that failed to produce resin and were successfully mass attacked at Lumby compared to Milk River may be a function of the high beetle pressure in that stand. Very rapid attack by a large number of beetles may have cut off translocation and interrupted the induced defense response before resin production was initiated. Because there was no significant difference in the variables describing attack between fast- and slow-growing trees in the same attack class (successful, light, or

unsuccessful attack), this suggests that once the defenses of the host had been overwhelmed, colonization was similar in both fast- and slow-growing trees. However, sample sizes within attack classes were small and unequal. The significantly higher density of attacks on successfully attacked trees at Lumby may also be related to the relatively high beetle pressure recorded at that site.

Conclusions

Although successful attacks were more commonly found in slow-growing trees (Chapter II), the results of this study show that western balsam bark beetle was capable of killing vigorously growing trees. However, the failure of western balsam bark beetle to induce mass attacks on a number of fast-growing trees, despite high population levels and the attraction of pheromone baits, may in part explain why mortality of subalpine fir caused by western balsam bark beetle was usually limited to slow-growing trees.

Chapter IV. Defense Response of Fast- and Slow-Growing Subalpine Fir to Inoculation with a Blue-Stain Fungus

Introduction

Bark beetles disseminate a variety of microorganisms, e.g., fungi, bacteria, protozoans, and yeasts, as they construct galleries in the host (Whitney 1982). Blue-stain fungi in the genera *Ceratocystis* and *Ophiostoma* are the most widely studied of these microorganisms. Although many bark beetles do not require blue-stain fungi to successfully colonize trees (Bridges *et al.* 1985), the fungi may contribute to tree death by killing tissues around beetle entrance holes and galleries, thereby interrupting translocation (Whitney 1982). When introduced at sufficiently high densities by artificial inoculations or unsuccessful beetle attacks, blue-stain fungi may be pathogenic to hosts without their beetle associates (Molnar 1965; Hornvedt *et al.* 1983; Christiansen and Solheim 1994; Solheim and Safranyik 1997; Krokene and Solheim 1998). Although blue-stain fungi vary in their level of pathogenicity, they elicit an induced defense response from the host and may be a potential tool for assaying host and stand resistance (Reid *et al.* 1967; Shrimpton and Reid 1973; Peterman 1977; Christiansen 1985; Miller *et al.* 1986; Raffa and Smalley 1988).

Fungal growth and development are inhibited in the host by the induced defense reaction. Nutrients essential to fungal growth and development are removed in lesion tissue by degenerative metabolism in cells, cellular necrosis (hypersensitivity), and localized secondary resinosis (Reid *et al.* 1967; Wong and Berryman 1977; Raffa and Berryman 1982b). Callus tissue or wound periderm then forms around the lesion, isolating the infected area from healthy tissue (Reid *et al.* 1967; Wong and Berryman 1977). Host resistance depends largely on the presence and strength of the induced response (Reid *et al.* 1967;

Shrimpton and Whitney 1968; Berryman 1972; Shrimpton 1973; Shrimpton and Reid 1973; Wong and Berryman 1977; Raffa and Berryman 1982b; Christiansen and Horntvedt 1983), and is related to host vigour (Chapter I; Berryman 1972, 1982a). The objective of this study was to examine the relationship between the induced defense response and host vigour in subalpine fir. Inoculation of subalpine fir with a blue-stain fungus associated with western balsam bark beetle was done to test the hypothesis that there is no difference in the rate and extent of the induced defense reaction in fast- and slow-growing trees.

Methods

Fungus Isolation[4]

A blue-stain fungus isolated from adult western balsam bark beetles attacking a subalpine fir bolt during the spring of 1999 in the Lumby area was used for this study. Fungal isolates were obtained from three adult beetles treated in the following manner: one beetle was set on a petri plate with 2% malt extract agar (MEA) and allowed to walk around for a day; a second beetle was macerated with 2 ml of sterile water; and a third beetle was placed in 2 ml of sterile water and shaken vigorously. Solutions from the last two beetles were diluted to ratios of 1:3, 1:30, and 1:300. One-tenth of a milliliter of each dilution was cultured on 2% MEA and 2% MEA amended with 100 ppm cycloheximide. All plates were incubated for 2 days at room temperature. Single-spore cultures were isolated from distinct colonies identified on the plates and incubated at room temperature on new plates (2% MEA). Seventeen of the 19 isolates were identified as being *Ophiostoma* fungi. The 2 remaining isolates may have been *Ophiostoma* fungi as they were growing on cycloheximide

[4] Isolations and descriptions of fungi in this section were provided by Adnan Uzunovic, Forintek Canada Corp., Vancouver, BC.

media, but they were not examined further. Thirteen of the *Ophiostoma* isolates, hereinafter referred to as *Pesotum A* (only asexual fruiting structures were observed), were dark brown with a growth rate of 3 mm per day on artificial medium and a pesotum anamorph. The remaining 4 *Ophiostoma* isolates, hereinafter referred to as *Pesotum B*, were lighter brown in color with a growth rate of 6 mm per day on artificial medium and no apparent anamorph. Neither isolate met the description of *O. dryocoetidis*, a pathogenic blue-stain fungus found to be associated with western balsam bark beetle (Kendrick and Molnar 1965). *Pesotum A* was selected for use in this study because it appeared in a greater number of isolations and was also present in a preliminary isolation from phloem tissue of a 1998 mass attack from Milk River. *Pesotum A* was subcultured and maintained on 1.25% MEA at room temperature.

Inoculum

Organic rye grains were inoculated with *Pesotum A* using a procedure adapted from Stamets and Chilton (1983). The rye grains were mixed with distilled water, at a ratio of 1:0.86, in a narrow-mouthed, quart-sized Mason jar. The jar was capped with a synthetic filter disc, which allowed for gaseous exchange to occur, but prevented the entry of contaminants. A plastic screw cap lid, with a 3/8" diameter hole drilled in the center, secured the filter disc in place. The jar was autoclaved for 1 hour at a temperature of 121°C and a pressure of 1.05 kilograms per square centimetre (approximately 1 bar, or 15 pounds per square inch). After removal from the autoclave, the jar was shaken to break up clumps of grains and allowed to cool to room temperature in a sterile environment. Small pieces of agar from an actively growing single spore isolate were added to the jar using sterile technique, and the jar was gently shaken to distribute them. The jar was stored at room

temperature in a clean cabinet and gently shaken every 3-5 days to redistribute fungal pieces and grains. Fungal colonization of grains was determined to be sufficient 10 days later, when mycelial growth was visible on grains throughout the jar. To facilitate inoculation in the field, grains were transferred to sterile 1.5 ml Eppendorf™ Disposable Biopur Safe-Lock Micro Centrifuge Tubes (Eppendorf Scientific, Hamburg, Germany), using sterile technique, one day prior to field use. Centrifuge tubes were stored overnight and transported to the field in a cooler. During the field inoculation procedure, one centrifuge tube was used per tree to reduce the chance of contamination.

Grains for control treatments were prepared as described above, except pieces of agar were not added to the jar.

Field Procedure

This study was conducted during the summer of 1999 at a site (UTM 5914800 654900), adjacent to the site referred to in Chapter II, in the Milk River Drainage, Robson Valley Forest District, British Columbia. The site was at an elevation of 1 050 m with an overall slope of less than 10% and occurred in the transition zone between the ESSFmm and the ICHmc1 biogeoclimatic zones (Meidinger *et al.* 1988).

Five 20 m radius plots were established throughout the stand to facilitate numbering and relocation of trees. All subalpine fir trees greater than 20 cm diameter at breast height occurring in the plots were numbered, and increment cores were extracted from the east and west aspects. Cores were stored at room temperature, mounted on wooden holders, and ring widths were measured using Windendro software (Regent Instruments Inc., Quebec City, QC, Canada) and a Hewlett-Packard ScanJet 4c/T scanner (Hewlett-Packard Ltd., Palo Alto, CA, USA). Age at breast height was measured on one core and measurements from both

cores were used to calculate the mean cumulative growth at breast height for the last 5 years (CUM5).

The mean CUM5 calculated for unattacked (control) trees, unsuccessful attacks, and successful attacks in Chapter II were considered when determining the limits of fast- and slow-growing categories. Because trees with a CUM5 of less than 2.8 mm (mean CUM5 of successful attacks calculated in Chapter II) were infrequent in the stand, the upper boundary of slow-growing trees was set at 3.2 mm. To maximize the differences in CUM5 between growth categories, the lower limit of fast-growing trees was set at 5.1 mm (mean CUM5 of control trees calculated in Chapter II was 4.5 mm). Trees with a mean CUM5 between 3.2 and 5.1 mm, or with cores that showed very different growth rates from one another, were excluded from the study. A total of 129 trees were cored in order to select 20 fast- and 20 slow-growing trees. The selected trees ranged in dbh from 20.5 to 48.5 cm and in age from 63 to 259 years.

The bole of each selected tree was brushed free of lichens and small twigs from 1.0 to 1.8 m above the ground. A small patch of bark was lightly flamed at six equal intervals around the bole at 1.4 m with a hand held propane torch. At each flamed spot a 4 mm diameter hole was punched to the outer surface of the sapwood with an ethanol-dipped and flamed leather punch (Arch Punch®, C.S. Osborne and Co., Harrison, NJ, USA). Two rye grains colonized by *Pesotum A* were placed in each hole with ethanol-dipped and flamed forceps. A sterile band-aid (Band-Aid®, Johnson & Johnson, North Brunswick, NJ, USA) was placed over the hole and then covered with duct tape to reduce the chance of contamination and desiccation. A second set of six holes was made at 1.15 m at staggered locations to the holes at 1.4 m. Two sterile rye grains (not colonized by fungus) were placed

in the holes to serve as controls. Control holes were constructed, filled and covered using the same method as for the fungus-inoculated holes. Treatments were applied on 3 August 1999, approximately 3 weeks after the main beetle flight in the area.

One randomly selected hole per treatment on each tree was examined at 3, 7, 10, 17, and 41 days following inoculation. The bark and phloem around the hole was peeled back with a buck knife or hatchet, taking care not to come in contact with the area of staining in the sapwood and phloem. The vertical and lateral extents of the lesion were measured using the outermost discolored edge on the sapwood surface as the boundary. The average daily growth rate of the lesion was calculated between each sample time. Two slivers of phloem and one sliver of sapwood were taken with an ethanol-dipped and flamed scalpel from the stained area adjacent to the inoculation hole and cultured on plates with 1.25% MEA amended with 100 ppm cycloheximide. Plates were stored for 10 days at room temperature and then checked for the presence of *Pesotum A* and contaminates.

Data Analysis

Variables were visually assessed for normality using histograms. No data required transformation to meet the assumptions of the statistical tests. A repeated measures general linear model (GLM) multivariate analysis of variance (MANOVA) using SYSTAT 9.0 (SPSS Inc., Chicago, IL, USA) was used to test for effects of inoculation, tree growth, and time on the length and width of the lesion. The repeated measures GLM-MANOVA provided univariate output for the between subjects effects and both univariate and multivariate outputs for the within subjects effects. The multivariate output was selected over the univariate split-plot analysis of variance (ANOVA) approach for interpreting within subjects effects as it requires fewer assumptions about homogeneity of variance and

covariance across trials and treatments and is generally preferred (Zar 1999; Tabachnick and Fidell 1996). Hypotheses tests were run after the GLM to test the specific effects of inoculation treatment and tree growth at each sample time on the length and width of the lesion.

Results

There was no significant difference between the mean dbh of fast- versus slow-growing trees ($t_{36,2}$ = -0.2, P = 0.841) used in the study. Mean (±SE) tree age at breast height was significantly greater in slow-growing trees than in fast-growing trees at 143.06 (±6.90) and 106.11 (±4.72) years respectively ($t_{28,5}$ = -3.076, P = 0.005).

Fungus Reisolation and Contamination of Cultures

During the study period, *Pesotum A* was successfully reisolated from fungus treatments on fast- and slow-growing trees 100% of the time, except on two occasions: 1 reisolation at both 7 and 10 days after inoculation failed to produce *Pesotum A*. Data from these 2 samples were excluded from the study. *Pesotum A* was cultured from a total of 20 control samples during the study (Table 10). The number of controls that produced *Pesotum A* increased over the study period (Table 10), however individual trees with controls that produced *Pesotum A* varied between sample periods. Fast- and slow-growing trees had similar numbers of controls contaminated with *Pesotum A*, but the observation size was small (Table 10). Data from controls that produced *Pesotum A* were excluded from the study. Due to excluded data, the number of observations in each treatment group (fast-growing fungus-inoculated, fast-growing control, slow-growing fungus-inoculated, and slow-growing control) ranged between 15 and 20 for a given sample period.

Contamination of reisolation cultures by organisms other than *Pesotum A*, e.g., bacteria, moulds, yeasts and various other fungi, ranged from 30 to 100% depending on sample period and treatment group (Table 11). Contamination of plates from all treatment groups remained steady throughout the study period varying from 51 to 56% except at the last sample time when the rate of contamination jumped to 78%. Plates with samples from controls were more likely to be contaminated than plates with samples from fungus-inoculations regardless of tree growth ($\chi^2_1 = 35.474$, $P < 0.001$) (Table 11).

Table 10. Number of reisolates from control treatments that produced *Pesotum A* for fast- and slow-growing trees.

	Days Post Inoculation					
	3	7	10	17	41	Total
Fast Control	0	2	1	2	5	10
Slow Control	0	0	0	5	5	10
Total	0	2	1	7	10	20

Table 11. Percent of reisolates contaminated with other fungi, moulds, bacteria, and/or yeasts at each sample time by treatment group.

	Days Post Inoculation					
	3	7	10	17	41	Total
Fast Control	60	65	65	70	95	71
Fast Fungus	50	45	40	30	80	49
Slow Control	70	70	65	75	100	76
Slow Fungus	40	45	50	30	40	41
Average	55	56	55	51	78	60

Lesion Morphology

At the first sample time, 3 days after inoculation, discoloration of phloem and sapwood tissues around both fungus and control treatments on fast- and slow-growing trees was visible. The wound response visible on the sapwood surface consisted of a thin brown

ring immediately around the inoculation hole, surrounded by a lighter colored area bordered by varying degrees of brown discolored tissue (Figure 5). The brown discoloration was generally more pronounced, in terms of size and darkness, in fungus versus control treatments for the duration of the study (Figure 6). At the second sample time, 7 days after inoculation, a large light colored region extended beyond the darkened tissue (Figure 5). Henceforth, the smaller dark colored area will be referred to as the dark area, and the larger light colored area will be referred to as the light area. At the fourth sample time, 17 days after inoculation, dark staining beyond the original boundaries of the dark area, but below the sapwood surface was observed for some fungus treatments (Figure 5).

Effects of Inoculation and Tree Growth on Rate and Size of Dark and Light Areas

The vertical and lateral growth curves of the dark and light areas for fungus and control treatments on fast- and slow-growing trees were logistic; a period of exponential growth, followed by deceleration, and stabilization (Figures 7 and 8). The majority of vertical and lateral growth in the dark area occurred by the second sample time, 7 days after inoculation, for fungus and control treatments on fast- and slow-growing trees (Figures 7A and 8A). The majority of vertical growth in the light area occurred by the second sample time, 3 days after it first appeared, for control treatments on fast- and slow-growing trees (Figure 7B). Vertical growth of the light area continued until the fourth sample time, 14 days after it first appeared, for fungus treatments on fast- and slow-growing trees (Figure 7B). Lateral expansion of the light area stabilized by the second sample time, just 4 days after it first appeared, for fungus and control treatments in fast- and slow-growing trees (Figure 8B).

Figures 9 and 10 show the vertical and lateral growth rates between sample dates for the dark and light areas. Vertical and lateral growth rates of the dark area were greatest

between 0 and 3 days after inoculation and between 3 and 7 days for the light area, for both fungus and control treatments on fast- and slow-growing trees (Figures 9 and 10). Little difference was observed between the vertical growth rate between sample times of fungus versus control treatments or fast- versus slow-growing trees except between the first and second sample time (Figure 9A). Between 3 and 7 days after inoculation, vertical growth of the dark area was greater in fungus versus control treatments and in fast- versus slow-growing trees (Figure 9A). Vertical growth of the light area followed a similar pattern (Figure 9B). Differences in the lateral rates of growth of both the dark and light area were less pronounced (Figure 10).

Inoculation had a significant effect on the length and width of both dark and light areas (Table 12). Tree growth had a significant effect on the length of both dark and light areas, but no effect on their widths (Table 12). Time had a significant effect on the length and width of both dark and light areas (length dark: Wilk's $\lambda_{4,18} = 0.02$, $P < 0.001$; width dark: Wilk's $\lambda_{4,18} = 0.06$, $P < 0.001$; length light: Wilk's $\lambda_{3,18} = 0.38$, $P < 0.001$; width light: Wilk's $\lambda_{4,18} = 0.19$, $P < 0.001$). The hypothesis test for the effect of inoculation at each sample time revealed that it had a significant effect on the length and width of both dark and light areas at all sample times except the first one, 3 days after inoculation (Table 13). Tree growth had a significant effect on the lengths of the dark and light areas at 7, 10 and 17 days after inoculation, but no effect at 3 and 41 days after inoculation (Table 13).

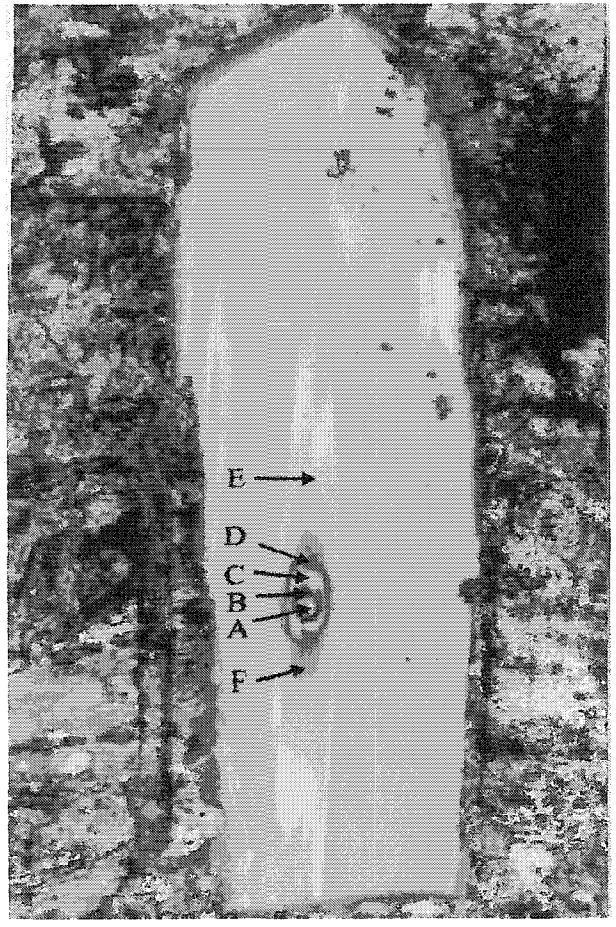

Figure 5. Morphology of the induced reaction 17 days after inoculation showing point of inoculation (A), ring of darkened tissue around inoculation hole (B), light area around inoculation hole (C), dark colored area (D), light colored, translucent looking area (E), and dark staining below sapwood surface (F).

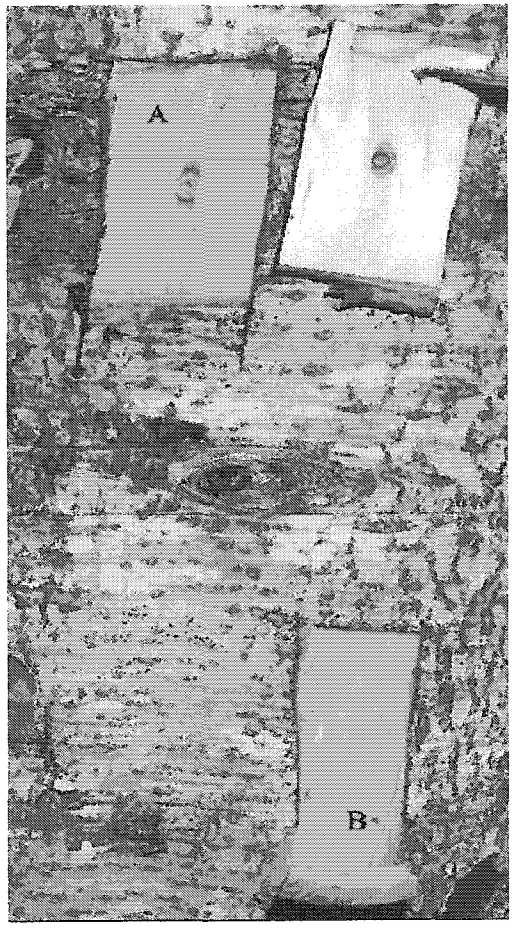

Figure 6. Comparison of the induced reaction 7 days after inoculation in response to fungus inoculation (A) and control treatment (B).

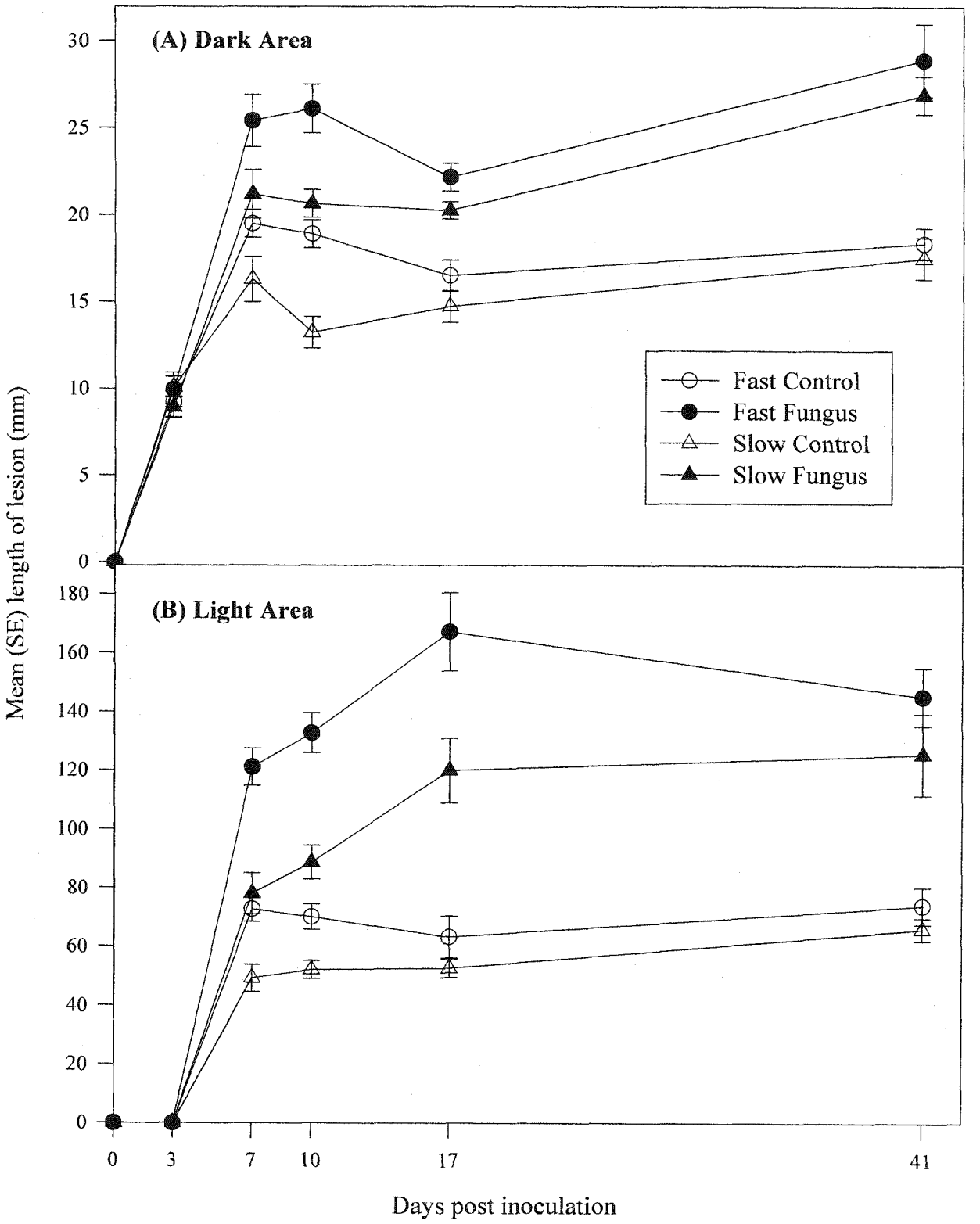

Figure 7. Comparison of mean (SE) lesion length on the sapwood surface at each sample time for fungus and control treatments on fast- and slow-growing trees for the dark and light area of the lesion.

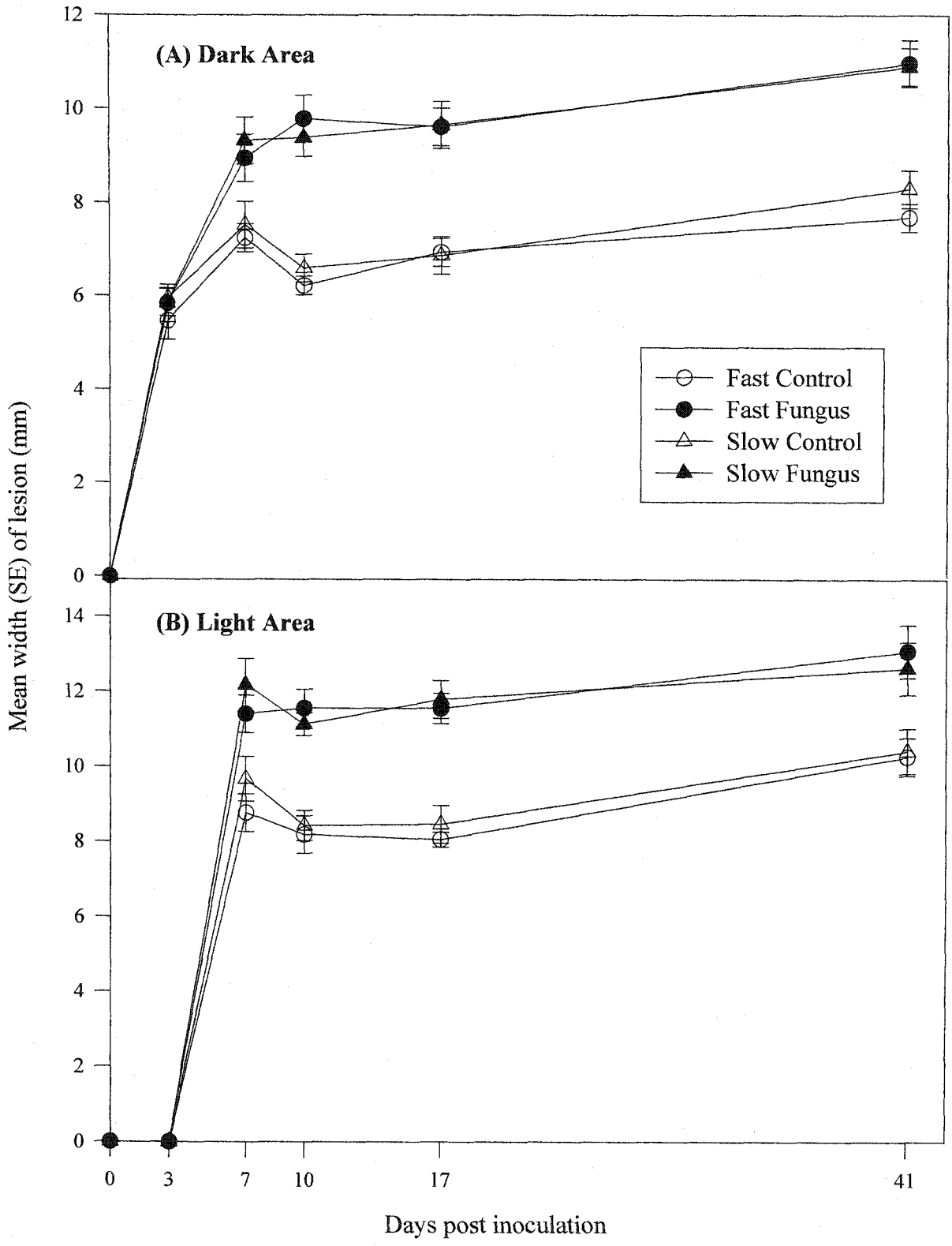

Figure 8. Comparison of mean (SE) lesion width on the sapwood surface at each sample time for fungus and control treatments on fast- and slow-growing trees for the dark and light area of the lesion.

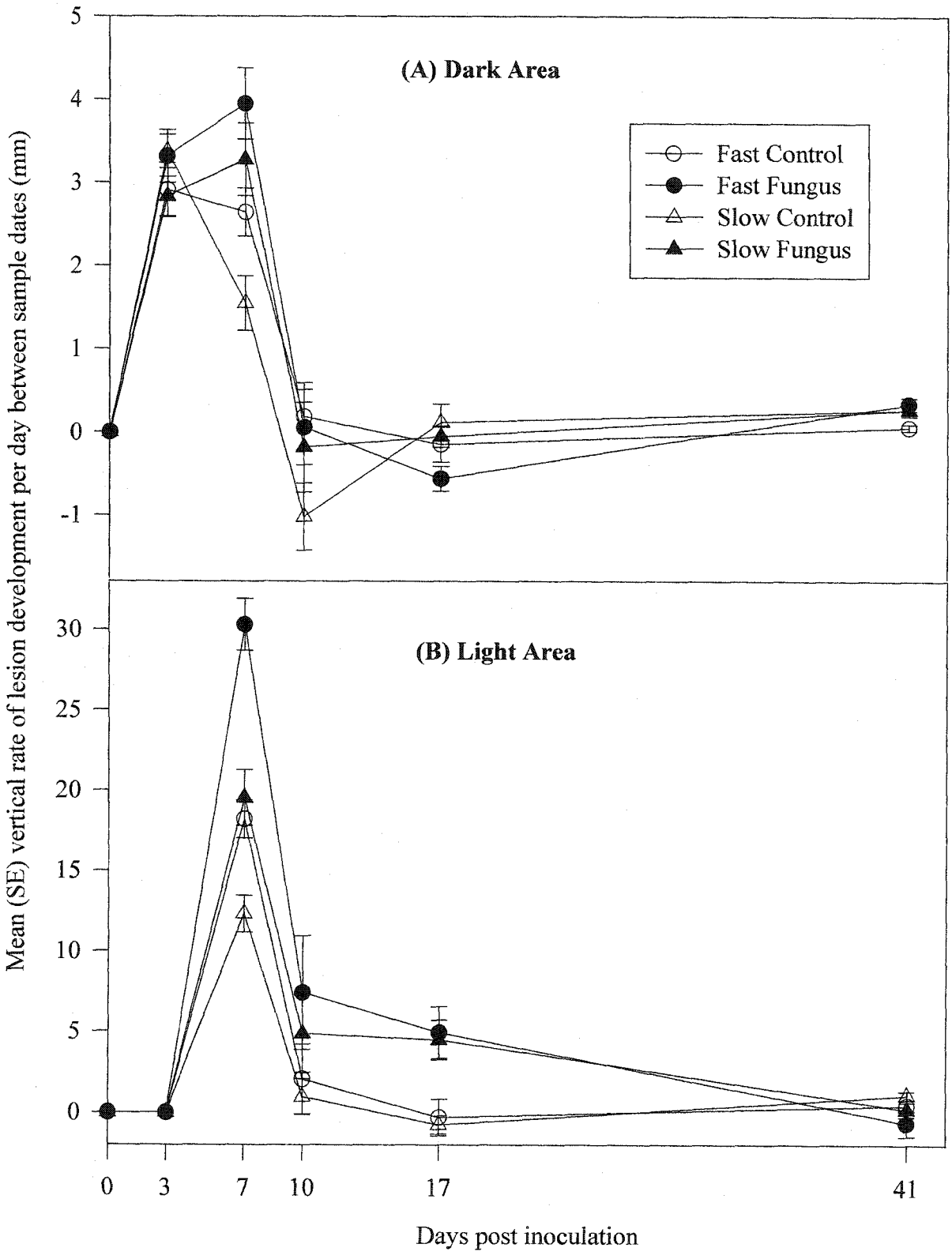

Figure 9. Comparison of mean (SE) vertical rate of lesion development per day between sample times for fungus and control treatments on fast- and slow-growing trees for the dark and light area of the lesion.

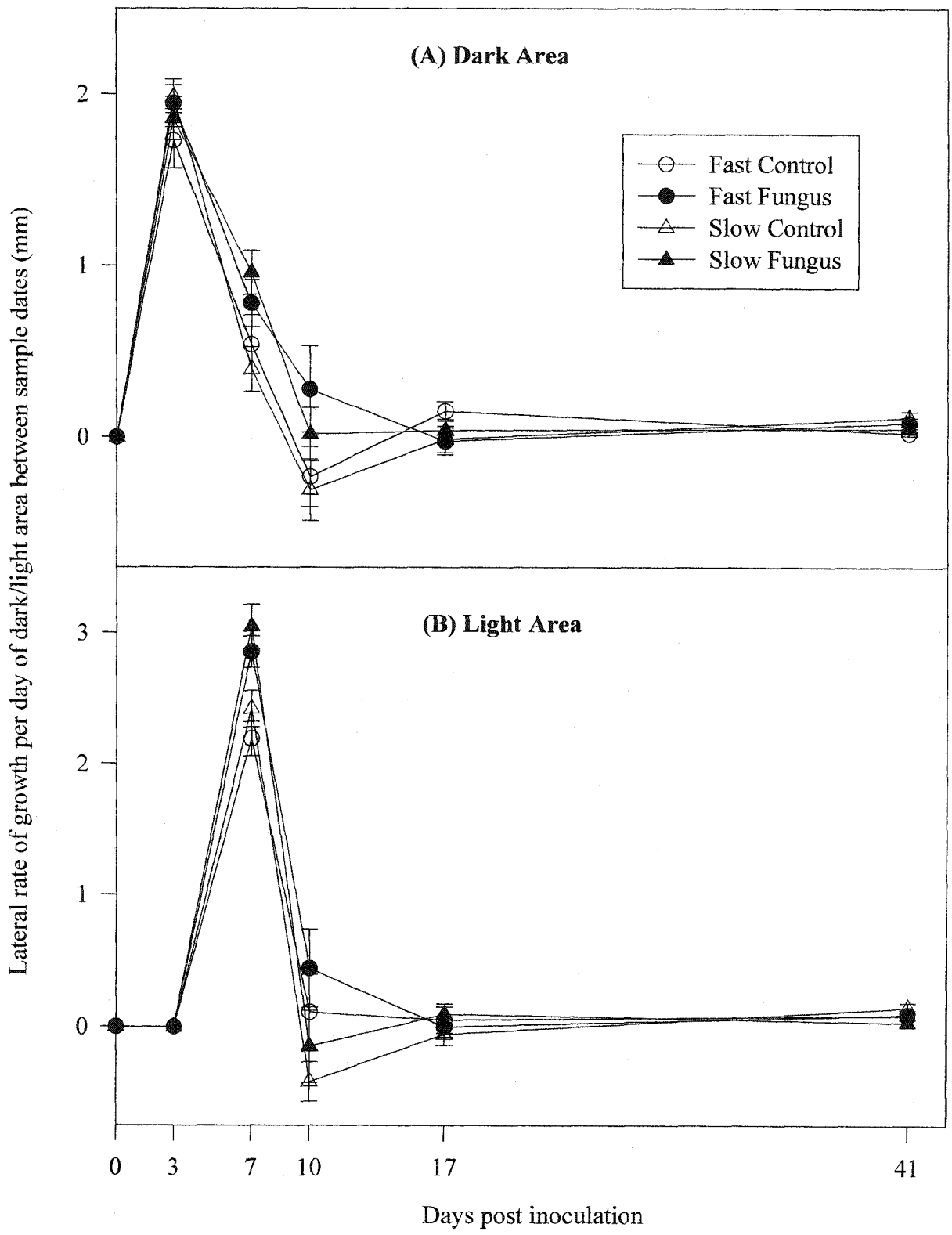

Figure 10. Comparison of mean (SE) lateral rate of lesion development per day between sample times for fungus and control treatments on fast- and slow- growing trees for the dark and light area of the lesion.

Table 12. ANOVA results for overall effects of inoculation treatment and tree growth on lengths and widths of dark and light areas.

	Length Dark Area			Width Dark Area			Length Light Area			Width Light Area		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
Inoculation	135.00	1,21	<0.001	122.43	1,21	<0.001	79.02	1,20	<0.001	62.18	1,20	<0.001
Tree Growth	10.59	1,35	0.003	0.08	1,35	0.782	17.91	1,34	<0.001	0.14	1,34	0.710

Table 13. Results of hypothesis testing for effects of inoculation treatment and tree growth on length and width of dark and light areas at each sample time.

		Days After Inoculation									
		3		7		10		17		41	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Length Dark	Inoculation*	0.55	0.466	22.45	<0.001	48.04	<0.001	71.12	<0.001	37.27	<0.001
	Tree Growth**	0.79	0.380	6.74	0.014	16.77	<0.001	6.08	0.019	2.00	0.166
Width Dark	Inoculation*	0.08	0.774	9.09	0.007	64.75	<0.001	38.54	<0.001	36.81	<0.001
	Tree Growth**	0.07	0.80	1.76	0.194	0.002	0.968	1.61	0.213	0.05	0.820
Length Light	Inoculation†	—	—	48.00	<0.001	65.60	<0.001	50.19	<0.001	30.19	<0.001
	Tree Growth‡	—	—	24.32	<0.001	22.01	<0.001	6.05	0.019	2.37	0.133
Width Light	Inoculation†	—	—	12.92	0.002	41.82	<0.001	54.18	<0.001	9.74	0.005
	Tree Growth‡	—	—	1.93	0.174	0.166	0.686	0.007	0.933	0.020	0.888

* df for all tests in this row are 1, 21.
** df for all tests in this row are 1, 35.
† df for all tests in this row are 1, 20.
‡ df for all tests in this row are 1, 34.

Discussion

A number of factors may have contributed to variation in the reisolation frequency of *Pesotum A* and contamination of cultures. The two times that *Pesotum A* was not reisolated from fungus treatments were likely due to the failure of the fungus to establish in the host. Host resistance, or competition with other invading organisms, may have prevented successful colonization of host tissue by *Pesotum A*. Small arthropods, e.g., mites, and Lepidoptera larvae, moving propagules from fungus to control treatments may have been responsible for the increased number of *Pesotum A* isolations from control treatments over time. Contaminants, e.g., bacteria, moulds, yeasts, and other fungi, may also have been introduced to fungus and control treatments by arthropods. In addition, contamination of both treatments may have occurred during or after inoculation by spores transported by wind or in water running down the trunk, as treatments were not hermetically sealed. The higher rate of contamination of control versus fungus treatments was likely due to competitive exclusion in the latter, where the grain was already colonized by *Pesotum A*.

Artificial inoculation with sterile grain and *Pesotum A* both led to the formation of lesions with similar morphologies. The progressive darkening of tissue around the wound site, large light streak, and induced resinosis are characteristic of wound responses elicited by artificial inoculation of other blue-stain fungi and control treatments in different tree species (Molnar 1965; Reid *et al.* 1967; Wong and Berryman 1977; Solheim 1988). The darkened tissues in wound responses are known to contain decreased sugar, starch, and moisture levels, but increased quantities of monoterpenes (Reid *et al.* 1967; Wright *et al.* 1979; Miller and Berryman 1982; Raffa and Berryman 1982b, 1983b; Raffa and Smalley 1995). The light

streak may be caused by embolism in the xylem from the severing of tracheids (Reid *et al.* 1967).

Although this study measured the lesion boundaries at the sapwood-phloem interface, the appearance of a larger area of darkened tissue below the sapwood surface indicates that the size of the reaction zone was greater below the surface. Maximum vertical penetration of certain blue-stain fungi on specific hosts has been observed below the sapwood surface (Christiansen and Solheim 1994; Uzunovic and Webber 1998). In contrast, radial sections through lesions caused by inoculation of *O. dryocoetidis* in subalpine fir resulted in phloem and cambial necrosis while sapwood remained unstained (Molnar 1965). However, in the same study inoculation of a less pathogenic blue-stain fungus that was also associated with western balsam bark beetle resulted in subsurface sapwood staining (Molnar 1965). Measurement of lesion boundaries at the sapwood surface may not be a good indication of the extent of the wound response.

The initial exponential growth of the lesion followed by a period of deceleration and stabilization was consistent with the development of fungus-induced lesions in other studies (Wong and Berryman 1977; Raffa and Berryman 1982b). The majority of lesion growth occurs almost immediately after inoculation, but induced resinosis was delayed while traumatic resin ducts form (Reid *et al.* 1967; Wong and Berryman 1977).

Although the induced defense system appears to be a generalized response to any incompatible invader or wounding, its intensity may vary. Inoculation with blue-stain fungi typically causes a more intense response, e.g., larger lesion size, greater concentration of monoterpenes, and increased resinosis, than sterile controls or mechanical wounding (Molnar 1965; Reid *et al.* 1967; Wong and Berryman 1977; Raffa and Berryman 1982b; Horntvedt *et*

al. 1983; Solheim 1988; Raffa and Smalley 1988; Solheim 1988; Lieutier *et al.* 1989; Parmeter *et al.* 1989). Although the mechanism of induction for the induced defense reaction is not well understood, studies indicate the intensity of the response may depend on an elicitor originating in phloem parenchyma cells (Paine *et al.* 1997; Franceschi *et al.* 2000). The elicitor, a diffusible chemical originating from the tree, fungus, or beetle, would transport a signal from damaged cells to surrounding cells (Lieutier 1993). The quantity of the elicitor released would determine the quantity of the resin produced, while the diffusibility of the elicitor would determine the length of the reaction zone (Lieutier 1993). The variation in intensity of the wound response may be due to the number of wounded cells involved and/or different elicitors associated with fungi and wounding. Inoculations with dead blue-stain inoculum and chitosan (fungal cell wall fragment) elicited larger responses than controls, suggesting that fungal cell wall fragments were involved in initiating the response (Miller *et al.* 1986; Raffa and Smalley 1988).

Because host vigour may affect host resistance, the factors that are thought to contribute to the induced defense reaction, e.g., current photosynthetic rate, transport of photosynthate to the wound site, synthesis of monoterpenes at the wound site, were expected to be superior in fast-growing trees compared to slow-growing trees. Fast-growing trees were expected to inhibit and contain fungal growth quicker, therefore producing smaller lesions than slow-growing trees. The similarity in lesion size on fast- and slow-growing trees 41 days after inoculation does not support this hypothesis. The assumption that fast-growing trees would respond quicker than slow-growing trees to invasion was not supported by sample results at 3 days after inoculation. However, larger lesions at 7, 10, and 17 days after inoculation in fast-growing trees may contribute to increased resistance. Because wound

periderm does not usually form until after fungal growth is slowed or arrested (Wong and Berryman 1977; Lieutier 1993), producing lesions fast, in order to contain the fungus while wound periderm forms, may be an advantage. Fast-growing trees may temporarily have lower translocation potential than slow-growing trees due to increased necrosis, but would improve their chances of containing the pathogenic invader. Alternatively, fungal growth may be related to physiological characteristics of fast-growing trees, e.g., larger cells or increased nutrition, resulting in increased fungal colonization and a larger reaction zone. However, the temporary difference in lesion size associated with tree vigour confounds this latter explanation.

The relationship between host resistance, vigour, and size of the reaction zone has been examined in a number of studies. Resistance of young Norway spruce (*Picea abies* (L.) Karst.) to *Ophiostoma polonicum* Siem., a pathogenic blue-stain fungus, was negatively correlated with lesion length, and percentage of cross-sectional area on tree disk that was blue-stained (Horntvedt *et al.* 1983; Christiansen 1985; Horntvedt 1988). No significant relationship and a positive correlation were found between resistance and growth indices in separate studies (Horntvedt *et al.* 1983; Christiansen 1985; Horntvedt 1988). Inoculations of the same fungus into subalpine fir did not produce significant differences in resistance between fast- and slow-growing provenances, or between trees with a high and low vigour index (basal area growth in last year/sapwood area) (Christiansen and Solheim 1990). Growth efficiency of Scots pine (*Pinus sylvestris* L.) was negatively correlated with length of the reaction zone induced by inoculation with *O. brunneo-ciliatum* Mathiesen-Käärik for both young and old trees (Lieutier and Ferrell 1988; Lieutier *et al.* 1993). Interpreting and comparing study results may be complicated by different definitions of resistance, vigour

indices, sample intervals, tree ages, methods of measuring lesion size, and tree or fungus species.

Lesion size (length or occluded sapwood area) has been used to assess the virulence of blue-stain fungi (Molnar 1965; Solheim 1988; Solheim and Safranyik 1997). More virulent fungi are expected to produce longer lesions because of increased colonization of host tissue, and/or invoking a stronger response in the host. However, differences in the habitat preferences of blue-stain species, e.g., sapwood or phloem tissue, which may also vary between tree species, confound comparisons and development of trends.

Conclusions

The stronger response of the trees to *Pesotum A* compared to the control inoculations, suggests that the fungus may play a role in eliciting the defense response of the host. However, the pathogenicity and virulence of *Pesotum A* are unknown at this time.

The temporary difference in the size of the induced reaction zone between fast- and slow-growing trees suggests that host vigour affects the induced defense response. If the induced defense response is weaker in slow-growing trees this may in part explain why successful attacks are more commonly found in slower-growing trees and why faster-growing trees are more likely to successfully defend themselves against attacks (Chapter II, III). Although the results of this study support the use of blue-stain fungi as a potential tool for assaying host resistance and comparing the induced defense response of trees, differences in induced defense reactions may be temporary and results may depend on the sample interval used.

Chapter V. Conclusions and Management Implications

Conclusions

This research identified six characteristics of subalpine fir that affect its susceptibility to western balsam bark beetle: diameter, age, radial growth in the last 5 years, proportion of bole with constant crown, crown volume, and of induced resinosis (Chapter II and III). Each of these characteristics may be related to host vigour. Because most other species of scolytids prefer low vigour hosts (Rudinsky 1962), the relationship between host vigour (as indicated by recent radial growth) and the induced defense response was examined further.

This research showed that host vigour, as indicated by recent radial growth, in part explains differences in the resistance of subalpine fir to western balsam bark beetle. Because subalpine fir lacks extensive primary resin canals, resistance depends on the induced defense response. Current literature suggests that the induced defense response involves three main processes: 1) cellular necrosis (hypersensitivity); 2) secondary resinosis; and 3) wound periderm or callus formation (Lieutier *et al.* 1993). Invading organisms are confined or slowed through the removal of nutrients by cellular necrosis, followed by resinosis. The formation of wound periderm then seals the infected area from healthy tissue. Because slow-growing trees were more likely to be selected for attack, and more likely to be successfully attacked than fast-growing trees (Chapter II), it was hypothesized that the induced defense response was less effective in slow- versus fast-growing trees. The greater incidence of induced resinosis on fast- versus slow-growing trees and the greater percentage of slow-growing trees that were successfully attacked support this hypothesis (Chapter II and III). The temporary difference in the

size of the wound lesion between fast- and slow-growing trees further suggests that the induced defense response is affected by host vigour (Chapter IV).

The preference of western balsam bark beetle for low vigour hosts may be related to its role in the ecosystem. Subalpine areas may be slow to regenerate after large scale disturbances and the fire return interval is likely large in such ecosystems (Jull 1983). In between large scale disturbance events, succession may be driven by processes working on a smaller scale throughout the stand, e.g., decay, rot, insects, and wind (Lewis and Lindgren 2000). These processes may work independently or in conjunction with other processes occurring in the stand. Because western balsam bark beetle selectively kills older, weakened trees, often in small patches, it aids in turning over the subalpine fir in these stands and creating small gaps in the forest canopy.

Species of bark beetles that rely on predisposing factors and have spatially and temporally defined populations, as opposed to species exhibiting eruptive behaviour, may be used as ecosystem indicators (Raffa 1995). Such species may be used to assay forest health in a changing environment. Fluctuations in population levels of western balsam bark beetle may be an indication of changes in the ecosystem. Population increases may reflect aging, decadent stands, or overstocked stands on poorer sites, while population decreases may indicate vigourously growing trees, or younger stands. A population decrease may also occur in an older stand, after western balsam bark beetle has removed the weaker, susceptible stems. Forest managers could use population levels and changes in population levels of western balsam bark beetle as indicators of stand condition and succession.

Future Research and Considerations

Although this research has identified a number of characteristics of susceptible hosts, a number of characteristics were not examined. The chemical composition of the induced resin and tissues in the wound reaction zone may affect the ability of a host to defend itself (Reid *et al.* 1967; Kosuge 1969; Wong and Berryman 1977; Bordasch and Berryman 1977; Wright *et al.* 1979; Miller and Berryman 1982; Raffa and Berryman 1982b, 1983b; Raffa *et al.* 1985; Lewinsohn *et al.* 1993; Raffa and Smalley 1995; Nagy *et al.* 2000). The effect of past unsuccessful attacks on host vigour and predisposition to attack in the future is unknown. Areas of necrotic tissue around unsuccessful attacks may weaken hosts, however, the host is capable of regenerating cambium over the wound. Microsite characteristics, e.g., moisture availability or brush height, may also affect host susceptibility.

Although a number of characteristics of susceptible hosts have been identified, their relationship with host vigour and the defense response needs to be examined further. Because the exact mechanics of the induced defense response have not been determined, the relationship between low vigour, which may be affected by age, slow-growth, and small crowns, and the defense response of the tree needs to be elucidated. Furthermore, the effect of heart rot on host vigour is unknown.

A greater understanding of the biology and ecology of western balsam bark beetle is also needed. More information on the basic biology of western balsam bark beetle, e.g., further elucidation of the timing and composition of the flight in different areas of the province, would contribute to the development and timing of management activities.

Little is known about the relationships between western balsam bark beetle, its associated microorganisms, and subalpine fir. Molnar (1965) reports that of 100 sampled dead subalpine fir attacked by western balsam bark beetle, only 35% of the mortality could be directly attributed to beetle damage (i.e. girdling by adults and larvae). Molnar (1965) concluded that mortality of the remaining 65 trees, which were unsuccessfully attacked by western balsam bark beetle, was likely caused by a fungal infection. Although numerous species of fungi were isolated from the necrotic lesions, *Ophiostoma dryocoetidis* was the most consistently isolated fungus associated with the condition and was determined to be pathogenic to subalpine fir (Molnar 1965). However, only 1 out of approximately 35 fader and red attacked trees that were felled during the summer of 1999, had only unsuccessful beetle attacks; the remaining trees all showed signs of successful beetle attacks (unpublished data). There may be different fungi associated with western balsam bark beetle in different geographic areas, or the relationship between *O. dryocoetidis* and western balsam bark beetle may have changed in the last 35 years. Furthermore, the relationship of western balsam bark beetle with its associated microorganisms may vary depending on population levels. Although *O. dryocoetidis* was not isolated during the course of this study, it may have been present as isolation attempts were limited and not exhaustive. Further research on the number, species, pathogenicity, and ecological role of microorganisms associated with western balsam bark beetle is needed.

Management Implications

In order to effectively manage insect populations to meet forestry objectives, it is important to understand the nature of host susceptibility. If we understand what makes a

tree/stand susceptible to attack, then we can develop techniques for the effective manipulation of insects, and manage stands to reduce their susceptibility.

Pheromone baiting heavily infested blocks on a grid pattern prior to harvest has been successfully employed to manage other tree-killing bark beetles, e.g., mountain pine beetle and spruce beetle. The goal of pheromone baiting is to attract beetles to specific trees. The benefits of this are twofold: first, local beetle populations are contained and concentrated protecting surrounding trees/stands from attack; and second, beetles are physically removed from the forest in baited trees that are harvested and taken for processing. Grid baiting blocks using bait centres of one or two trees is currently being employed in British Columbia as a management technique for western balsam bark beetle. Because pheromone baited slow-growing trees are more likely to be successfully attacked than pheromone baited fast-growing trees in stands with medium to high beetle populations, a greater number of beetles would be removed from the forest if less vigourous hosts were selected for baiting. Pheromone baited fast-growing trees may also be successfully attacked in stands with very high beetle populations, so the benefits of baiting less vigourous trees may not outweigh the costs of identifying such trees in these stands.

Pheromone baiting cutblocks prior to harvest is an effective management technique when localized populations of bark beetles can be identified, contained, and removed before they spread. Because the distribution of western balsam bark beetle is generally not as concentrated as other tree-killing bark beetles, pheromone baiting heavily infested blocks prior to harvesting may have little impact on mortality in surrounding stands if the beetle is established over a large area. Pheromone baits may

protect trees immediately adjacent to the block, but small patches of infested trees over the landscape will continue to provide a source of beetles. Baiting large areas and selective patch-cutting may be a possibility if the entire infested area is baited, and the baits prove effective in drawing beetles to the bait centers. If dispersing beetles attack naturally susceptible trees en route to a baited tree, significant residual populations of beetles may be left in the stand. Furthermore, the edges around small patch cuts may provide an increase in weakened or windblown trees, which are susceptible to western balsam bark beetle attack. The most effective approach for managing a widely distributed insect would be to manage stand susceptibility at the landscape level.

As western balsam bark beetle preferentially attacks weakened hosts and is less effective at overcoming the defenses of faster-growing trees, practices that increase host vigour will increase resistance. For example, thinning has been identified as a potential stand management technique for enhancing resistance to mountain pine beetle and spruce beetle by increasing host vigour (Mitchell *et al.* 1983; Hard 1985; Warring and Pitman 1985). Increased spacing also opens the canopy resulting in pheromone dispersal from greater air movement through the stand (Fares *et al.* 1980). This may interrupt secondary attraction, which western balsam bark beetle relies on for inducing mass attacks. Because western balsam bark beetle mortality often occurs in patches, thinning may further decrease the number of attacks. However, thinning operations could cause damage to residual trees, which may increase their susceptibility to decay and heart rot.

Managing subalpine stands based on age may also affect their susceptibility. As harvesting is occurring in many ESSF stands in British Columbia's interior for the first time, these stands may not have experienced a stand-replacing disturbance for hundreds

of years. Thus, dominant trees are old, and the incidence of rot and insects are high. If second generation stands are managed to maximize growth, trees will reach commercial size before they become decadent, and susceptibility to decay and insects should be reduced. Therefore, mortality caused by western balsam bark beetle should rarely reach levels of concern.

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Appendix 1: Pearson correlation matrices of growth indices of successful attacks, unsuccessful attacks, and controls by site.

Cherry Ridge (n=19)

	LYI	CUM5	CUM10	PGR5	PGR10	PGR5/45	BAI5	DBH/CUM5
LYI[†]	1.00[*]							
CUM5	0.93	1.00						
CUM10	0.91	0.96	1.00					
PGR5	0.48	0.57	0.33	1.00				
PGR10	0.60	0.79	0.66	0.74	1.00			
PGR5/45	0.70	0.83	0.68	0.82	0.94	1.00		
BAI5	0.89	0.93	0.97	0.30	0.59	0.63	1.00	
DBH/CUM5	-0.90	-0.86	-0.86	-0.45	-0.73	-0.68	0.81	1.00

[†] LYI, last year increment; CUM5, last 5 years cumulative growth; CUM10, last 10 years cumulative growth; PGR5, 5 year periodic growth rate; PGR10, 10 year periodic growth rate; PGR5/45, CUM5 divided by the cumulative growth of the 45 years before that; BAI5, basal area increment in the last 5 years; DBH/CUM5, diameter at breast height divided by CUM5.

[*] $r_{0.05[17]} = 0.46$, $r_{0.01[17]} = 0.58$.

Lumby (n = 33)

	LYI	CUM5	CUM10	PGR5	PGR10	PGR5/45	BAI5	DBH/CUM5
LYI	1.00[*]							
CUM5	0.86	1.00						
CUM10	0.80	0.97	1.00					
PGR5	0.62	0.61	0.42	1.00				
PGR10	0.67	0.74	0.70	0.58	1.00			
PGR5/45	0.80	0.89	0.83	0.67	0.87	1.00		
BAI5	0.71	0.89	0.85	0.60	0.60	0.73	1.00	
DBH/CUM5	-0.72	-0.86	-0.85	-0.61	-0.68	-0.80	0.72	1.00

[*] $r_{0.05[31]} = 0.34$, $r_{0.01[31]} = 0.44$.

Milk River (n=22)

	LYI	CUM5	CUM10	PGR5	PGR10	PGR5/45	BAI5	DBH/CUM5
LYI	1.00[*]							
CUM5	0.95	1.00						
CUM10	0.87	0.96	1.00					
PGR5	0.58	0.53	0.28	1.00				
PGR10	0.64	0.65	0.60	0.47	1.00			
PGR5/45	0.76	0.79	0.67	0.73	0.85	1.00		
BAI5	0.84	0.88	0.78	0.67	0.48	0.69	1.00	
DBH/CUM5	-0.85	-0.92	-0.90	-0.49	-0.61	-0.78	-0.76	1.00

[*] $r_{0.05[19]} = 0.43$, $r_{0.01[19]} = 0.55$.

Appendix 2: Pearson correlation matrices of tree characteristics of successful attacks, unsuccessful attacks, and controls by site.

Cherry Ridge (n=20, except correlations with Age, n=15, CNAGE, n=16, or Age correlated with CNAGE, n=13)

	DBH	Height	Age	CNAGE	CUM5	CRTTL	CRCNT	AVCRWD	CRVOL	AVPHLM
DBH[†]	1.00[*]									
Height	0.77	1.00								
Age at 0.5 m	0.46	0.31	1.00							
CNAGE	0.60	0.51	0.14	1.00						
CUM5	-0.21	-0.16	-0.46	0.02	1.00					
CRTTL	-0.28	-0.04	-0.13	0.06	0.62	1.00				
CRCNT	-0.04	0.08	-0.05	0.05	0.68	0.68	1.00			
AVCRWD	0.58	0.40	0.22	0.40	0.26	0.21	0.36	1.00		
CRVOL	0.49	0.49	0.11	0.26	0.40	0.43	0.62	0.90	1.00	
AVPHLM	0.67	0.57	0.27	0.27	0.13	0.02	0.28	0.60	0.66	1.00

[†] DBH, diameter at breast height (1.3 m); CNAGE, canopy age; CUM5, recent 5 year cumulative growth; CRTTL, percent of bole with live crown; CRCNT, percent of bole with constant crown; AVCRWD, crown width; CRVOL, crown volume; and AVPHLM, average phloem thickness.

[*] $r_{0.05[18]} = 0.44$, $r_{0.05[13]} = 0.51$, $r_{0.05[14]} = 0.50$, $r_{0.05[11]} = 0.55$, $r_{0.01[18]} = 0.56$, $r_{0.01[13]} = 0.64$, $r_{0.01[14]} = 0.62$, $r_{0.01[11]} = 0.68$.

Lumby (n=33, except correlations with Age, n=29, or CNAGE, n=31)

	DBH	Height	Age	CNAGE	CUM5	CRTTL	CRCNT	AVCRWD	CRVOL	AVPHLM
DBH	1.00[*]									
Height	0.72	1.00								
Age at 0.5 m	0.25	0.26	1.00							
CNAGE	0.19	0.26	0.06	1.00						
CUM5	-0.03	-0.02	-0.14	-0.31	1.00					
CRTTL	0.18	0.10	-0.05	-0.23	0.17	1.00				
CRCNT	0.16	0.20	-0.12	-0.10	0.12	0.66	1.00			
AVCRWD	0.52	0.33	0.09	-0.13	0.24	0.35	0.21	1.00		
CRVOL	0.62	0.52	0.07	-0.01	0.18	0.46	0.42	0.93	1.00	
AVPHLM	0.64	0.48	0.14	0.21	0.09	-0.06	0.02	0.48	0.55	1.00

[*] $r_{0.05[31]} = 0.34$, $r_{0.05[27]} = 0.37$, $r_{0.05[29]} = 0.36$, $r_{0.01[31]} = 0.44$, $r_{0.01[27]} = 0.47$, $r_{0.01[29]} = 0.46$.

Appendix 2 (cont'd.)

Milk River (n=22, except correlations with Age, n=18)

	DBH	Height	Age	CNAGE	CUM5	CRTTL	CRCNT	AVCRWD	CRVOL	AVPHLM
DBH	1.00*									
Height	0.57	1.00								
Age at 1.3 m	0.67	0.28	1.00							
CNAGE	0.13	0.23	0.34	1.00						
CUM5	-0.02	0.14	-0.31	-0.11	1.00					
CRTTL	0.50	0.26	0.37	0.27	0.04	1.00				
CRCNT	0.31	0.44	0.02	-0.10	0.19	0.48	1.00			
AVCRWD	0.31	0.13	0.17	0.02	-0.17	0.55	0.08	1.00		
CRVOL	0.52	0.47	0.32	<0.01	-0.09	0.67	0.56	0.83	1.00	
AVPHLM	0.56	0.33	0.60	0.02	-0.12	-0.04	-0.10	-0.10	-0.02	1.00

* $r_{0.05[20]} = 0.42$, $r_{0.05[16]} = 0.47$, $r_{0.01[20]} = 0.54$, $r_{0.01[16]} = 0.59$.

Appendix 3: A comparison of tree characteristics between sites.

	Cherry Ridge		Lumby		Milk River	
	n	Mean (SE)*	*n*	Mean (SE)	*n*	Mean (SE)
Diameter (cm)	21	39.1 (1.6)*a*	33	26.7 (0.9)*b*	22	39.3 (1.7)*a*
Height (m)	21	27.7 (0.6)*a*	33	21.4 (0.5)*b*	22	25.5 (0.6)*c*
Age	15	167.0 (13.3)*a*	29	230.9 (7.0)*b*	18	159.3 (5.3)*a*
Canopy Age	16	120.3 (1.4)*a*	31	114.8 (2.0)*a*	22	122.5 (5.1)*a*
5 year growth (mm)	20	3.8 (0.6)*a*	33	3.6 (0.3)*a*	22	3.8 (0.3)*a*
% of bole with live crown	21	68.4 (3.5)*a*	33	64.0 (1.8)*a*	22	69.7 (2.5)*a*
% of bole with constant crown	21	53.0 (2.3)*a*	33	54.0 (1.8)*a*	22	59.9 (2.0)*a*
Mean crown width (m)	21	3.1 (0.1)*a*	33	2.5 (0.1)*b*	22	3.6 (0.1)*a*
Crown volume (m^3)	21	41.0 (4.7)*a*	33	19.7 (1.9)*b*	22	47.7 (4.4)*c*
Mean phloem width (mm)	20	6.2 (0.3)*a*	33	4.5 (0.2)*b*	22	6.3 (0.2)*a*

* Means within each variable followed by the same letter were not significantly different ANOVA, $P > 0.05$. Significant ANOVAs were followed by Bonferroni MCP, significant if $P \leq 0.05$. Crown volume was transformed to a logarithm to correct for non-normality.