Why Burning Brings Beetles: Fire-Bark Beetle Interactions

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Executive Summary and Deliverables

This project was undertaken to continue monitoring of old ponderosa pine trees prescribed burned in an earlier Joint Fire Science Program project, and to examine the physiological mechanisms behind increased bark beetle attack on ponderosa pine. We designed six experiments to meet these objectives.

Experiment 1. We evaluated vigor and survivorship of 1725 old ponderosa pine at Crater Lake National Park, Oregon that had been burned in spring and fall prescribed fires in 2002. By fall of 2006, 139 pines had died, representing 8.1% of the population. Twenty-four of these died directly due to burning or windthrow in 2002-2003. The remaining mortality was primarily from bark beetles. Control (no-burn) units had the least mortality (2.3%); spring burning increased mortality (6.1%), and fall burning increased mortality further (16.4%), due to higher intensity fires in the fall. Mortality was highest in post-fire year 1, and declined over time. Mortality was highest in the lowest vigor classes, and higher annual rings per cm (lower growth rate) were correlated with lower crown vigor. By 2005, fuels created by the burning had resulted in recovery of 84% and 71% of fine and total fuel loads, comparable figures for fall burns were 60% and 55%. Thus, a second treatment may be needed to reduce these fuels, but will likely not create the same dead fuel loads as the first burns did.

Experiment 2. We evaluated resin flow in 90 of the trees burned in Experiment 1 across a suite of vigor classes for five years. Trees in both spring and fall burns had higher resin flow than control trees, and the effect was maximized in the second post-fire year. By year 5, resin flows in burn units were similar to control trees.

Experiment 3. In an effort to partition the effects of fire on burned trees, we implemented a number of fire and fire surrogate treatments on trees at Sun Pass State Forest, Oregon. Pruning to simulate crown damage, two root plowing treatments to simulate root damage, a burn treatment, and a control treatment were used (5 trees each), and resin flow (an indicator of resistance to beetle attack) was followed. Trees in the burn treatment had immediately less resin flow than the control trees. Later, the burn treatment trees had higher resin flow, while other fire surrogate treatments were more similar to control trees.

Experiment 4. In an attempt to further understand the effect of fire on resin flow, we heated tree boles only, using charcoal fires burning around small platforms surrounding the tree bole. Post-treatment resin flow indicated that burned trees had lower resin flow than unburned (control) trees one month after burning, but that burned trees had higher resin flow (and similar flow to control trees) one year after burning.

Experiment 5. Resin chemistry has been identified as a significant factor in the susceptibility of trees to beetle attack. We evaluated monoterpene chemistry of 46 trees at two locations: Okanogan-Wenatchee National Forest, WA and Sun Pass State Forest, OR. Of the seven monoterpenes identified, few patterns existed between burned trees and control trees.

Experiment 6. Because we were repeatedly sampling the same trees for resin flow, we developed a unique sampling technique, and compared it to the more typical arch punch

technique that is commonly used. We found resin flow measurements were similar between the two methods.

Overall, mortality of old pines at Crater Lake stabilized after 4 years, and mortality was most highly correlated to season of burning, with higher intensity burns in the fall. Focusing on spring burns or very late season fall burns may be an appropriate way to continue the burning programs without undue mortality of old pines. Second burns may be necessary soon (5-7 years later) to clean up the dead fuels created by the first burns.

Resin flow in burned trees appears to decline immediately after burning but is higher in several post-fire years than control trees. This short-term decline does not appear to be related to beetle attacks. Post-fire increases in resin flow appear to be related to bole injury more than crown or root injury. Increased resin flow appears to be related to higher mortality on older ponderosa pine, undermining the conventional belief that resin defenses are indicative of survivorship. Survivorship appears more closely related to high radial growth, high crown vigor, and lower fireline intensities that do less damage to tree boles.

Deliverables

Proposed	Delivered	Status
Continue monitoring of 1725 old pines at Crater Lake	This report	Done
Final report to JFSP	This report	Done
Ph.D. dissertation of Daniel Perrakis	In progress	Autumn 2008
Two journal articles	In progress	Will follow dissertation completion
Workshop and Manager report	Held in 2006, Sisters, OR	Done - See appendix 2 in this report
Short courses	Technical Fire Management	Done in 2005-2008
JSFP Annual Reports	Annually	Done

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Appendix 1. Latin and common names of species.

Appendix 2. Perrakis, D.D.B., and J.K. Agee. 2006. Seasonal fire effects on mixed-conifer forest structure and ponderosa pine resin properties. Canadian Journal of Forest Research **36**:238-254.

Appendix 3. Kolb, T.E., J.K. Agee, P.Z. Fulé, N.G. McDowell, K. Pearson, A. Sala, and R.H. Waring. 2007. Perpetuating old ponderosa pine. Forest Ecology and Management **249**:141-157.

Introduction

Fire is recognized as a defining disturbance agent in dry forests of the western US. Consequently, the fire exclusion paradigm has been shown to be a flawed management strategy, and various restoration treatments are now commonly applied to forestlands as part of restoration and community protection efforts. Although such treatments have often been successful, the specifics are still being fine-tuned in many areas to match restoration objectives. Stand-level silvicultural methods and various types of prescribed burning are the restoration tools most frequently employed in these fire restoration efforts.

At Crater Lake National Park, such efforts have faced recurring problems related to post-fire insect outbreaks, severely undermining stand-level objectives. The main difficulties lie with poor forest health conditions and low individual tree resilience. Restoration treatments appear to have "shock" effects that can lead to high mortality among trees in forests being restored. For those individuals that survive the treatments in the short term, weakened defenses and complex interactions with insects and pathogens make long-term survival uncertain. Despite considerable study, there are still many knowledge gaps relating to physiological interactions between fire injuries, tree defense mechanisms, insect host-selection, and recovery through time.

Bark beetles[1] are known to interact with fire in many ways (McCullough et al. 1998), although some of the interaction mechanisms are only beginning to be understood. In ponderosa pine forests, deadly attacks by insects such as *Dendroctonus* beetles appear to increase after prescribed burns or wildfires (Miller and Keen 1960, Ganz et al. 2003, McHugh et al. 2003, Perrakis 2004, Breece et al. 2008). Burning treatments are often designed and implemented to protect dominant ponderosa pines from competing fire-intolerant vegetation. High beetle-related

[1] Common names are used for most biota in this text. A key to the Latin and common names for all species mentioned is provided in Appendix 1.

mortality after fire in these trees is therefore a critical management issue in many areas (Kolb et al. 2007).

One of the main difficulties in addressing this topic is in the complexity of the disturbance mechanisms affecting both trees and beetles. A comprehensive understanding of the issue necessitates merging literature from the fields of forest ecology, entomology, and, at the individual tree and tissue level, plant physiology and even organismal and molecular biology. Interest in understanding these systems and interactions is not new (e.g. Miller and Keen 1960, Wagener 1961). Early work conducted decades ago yielded important breakthroughs in many areas, especially in the areas of bark beetle host-selection mechanisms and pheromones. However, the search for broad similarities between different species (of both beetles and host trees, see Wood 1972, Christiansen et al. 1987) has revealed some fundamental differences in interaction mechanisms; despite researchers' hopes, bark beetle-host tree relationships are not all created equal. Furthermore, differences in research methods, overly confident and sweeping conclusions, and inconsistent use of terminology have led to confusion and interpretation errors.

Fire restoration of ponderosa pine forests in Crater Lake National Park, southern Oregon, began in the late 1970s. Following a few years of experimental prescribed burning, problems with post-fire tree mortality attributed to beetles were identified in the mid 1980s (Thomas 1982, Thomas and Agee 1986). While overall understanding of the system has increased over the years, viable management solutions to the restoration problem remain elusive. A few research projects attempted to answer some of the larger questions related to mortality mechanisms. These took the form of empirical approaches based on comparatively simple hypotheses (e.g. Swezy and Agee 1991, Feeney et al. 1998), but did not adequately explain interactions between prescribed fire and beetle attacks. For the present study, a more in-depth approach was taken,

using a longer time scale and multiple physiology-based experiments informed by emerging autecology theories. Thus, the purpose of this project was to examine fire effects on bark beetle susceptibility from a defense physiology perspective, with a number of related experiments aimed at filling in knowledge gaps at various scales of analysis.

The modern era of prescribed burning at Crater Lake National Park, Oregon, began over 25 years ago (Swezy 1988). Restoration efforts, including prescribed fire, were initiated after a commissioned fire history study suggested that the park's ponderosa pine stands were at risk of disappearing completely; the absence of periodic fire promoted encroachment by white fir and other species, which were then able to outcompete the pines (McNeil and Zobel 1980). The southeastern region of the park consists of old-growth mixed-conifer/ponderosa pine forest with a low-severity fire regime (*sensu* Agee 1993; McNeil and Zobel 1980), and is representative of widely distributed mixed-conifer forests elsewhere in Oregon and California. At Crater Lake, no fires burned in these forests between park establishment in the early 20th century until prescribed burning began in the late 1970's. Lightning-caused or accidental human ignitions during that time were all promptly extinguished. Goals of the modern fire program include killing post-settlement understory trees, maintaining the dominant pine cohort, and reducing fuel loads. In the past decade, these objectives have been refined and quantified, and now include forest structure and fuels specifications designed to meet restoration timelines and post-treatment wildfire intensity targets (Anonymous 2002).

Following the first decade of burning projects, problems with the restoration treatments became apparent. Specifically, canopy-dominant ponderosa and sugar pines were dying in the first few years following fires. Mortality of these trees continued for several years after burning, and were appeared to be partly caused by bark beetle attacks, especially the western pine beetle

(attacking ponderosa pine) and mountain pine beetle (attacking sugar pine) (Thomas and Agee 1986, Swezy and Agee 1991, Agee 2003). A recent Joint Fire Science Program study (00-2-31) on seasonal burning at Crater Lake observed a similar response, noting higher mortality of large ponderosa pines following hot fall burns than following cool spring burns, continued mortality each year after prescribed fire treatments, and post-fire beetle attacks playing a dominant role in ponderosa pine mortality (Perrakis 2004, Perrakis and Agee 2006 --see Appendix 2). A major component of that study examined ponderosa pine resin defenses, seeking to identify whether fire might reduce resin flow or pressure; no evidence of such a mechanism was detected, with many burned trees showing stronger resin defenses than unburned controls. These findings suggested some notable inconsistencies in the state of knowledge of fire-beetle interactions. In the absence of an obvious restoration strategy, and facing a lack of understanding of the processes at work, fire restoration in the Crater Lake panhandle is presently suspended (M. Murray, Crater Lake NP Terrestrial Ecologist, personal communication, 2007). These factors provided the impetus for the current project.

This final report summarizes the findings made in the Ph.D. dissertation of Daniel Perrakis which will be filed with the University of Washington in Autumn 2008. Detail concerning statistical tests and tables are contained in the dissertation. This report also contains an appendix that summarizes a workshop held in Sisters, Oregon in Summer 2006 (part of this JFSP project) to discuss unanticipated mortality of old ponderosa pine. Following the workshop, which included representatives from 12 agencies and institutions, several participants wrote a review paper on perpetuating old ponderosa pine (Kolb et al. 2007) and this paper is included as Appendix 3 to this report.

Literature Review

Bark beetle life history characteristics and ecology are well described elsewhere in the literature (see Miller and Keen 1960, Rudinsky 1962, Wood 1972, 1982, Christiansen et al. 1987, Schowalter and Filip 1993). A brief synopsis of a typical western pine beetle attack sequence will be useful for the discussion that follows. Wood (1972) defined the four stages of attack as dispersal, host selection, concentration and establishment. Dispersal is accomplished by flight and appears temperature-dependent, although little is known about distances covered or the duration of travel by individual beetles. Host selection has been a contentious topic among researchers, but is believed to be accomplished via either random landings independent of species and host suitability, or by primary attraction to chemical odors released by potential hosts.

Western pine beetles parasitize large (40+ cm dbh), typically old, low vigor ponderosa pines. Upon location of a suitable potential host, beetles immediately begin feeding and nesting activities in the bark, and release powerful attractant pheromones that attract other beetles to the focus tree in a mass attack. During the establishment phase, beetles dig egg galleries and oviposit in the phloem. In the spring, emerging larvae feed on the phloem and outer bark before emerging and dispersing, completing the life cycle. The host tree is killed due to phloem girdling from the larval galleries as well as disruption of water transport by blue-stain fungi (symbiotic with the beetles) that grow in the sapwood tracheids (Miller and Keen 1960; Wood 1972). Although many of these steps are similar for other species of *Scolytidae*, others are not, and thus inference to the Crater Lake-ponderosa pine case from studies on different host or insect species should be viewed with some caution.

Initial host-selection by bark beetles therefore occurs via one of two methods: random dispersal, or primary attraction (Wood 1982). The latter (Moeck et al. 1981) refers to the phenomenon of beetles being attracted from afar to chemical cues emitted by potential host trees (kairomones; Wood 1982). However, despite extensive research, interest, and the suspicions of many entomologists (R. Gara, personal communication 2005; A. Eglitis, USDA Forest Service entomologist, personal communication 2006, Lorraine McLaughlan, BC Forest Service, personal communication 2007), no conclusive evidence has confirmed primary attraction between western pine beetles and ponderosa pines under field conditions (Wood 1982, Raffa et al. 1993). Excluding the possibility of primary attraction, the default host-selection mechanism is random landing, independent of tree species or host suitability (Wood 1972, Raffa et al. 1993). In general, initial host selection has proven to be a challenge to study (due to the rarity of observing adult beetles in flight, and the confounding effects of attraction pheromones following establishment), leading some entomologists to believe that the lack of evidence for primary attraction merely reflects the failures of researchers thus far to design conclusive experiments on the topic.

Numerous studies examining fire-bark beetle interactions have observed elevated conifer mortality attributed to bark beetles following fire, compared with unburned controls. However, the mechanism behind this mortality increase is not obvious, with at least two obvious possibilities. The first possibility is an elevated number of initial beetle attacks, and no reduction in tree defenses; a second possible mechanism is the converse – no increase in beetle attacks, but a reduction in tree defenses. The two mechanisms might also occur together: both increased initial attacks and reduced host tree defenses.

The primary conifer defense against bark insects is from oleoresin (or simply resin, Phillips and Croteau 1999). Resin is considered a secretion, or a metabolic end-product substance that is not remobilization for other purposes. Accordingly, its only purposes appear to be defense against pathogens or desiccation, or wound healing (Fahn 1979, Himejima et al. 1992, Kozlowski and Pallardy 1997b). Higher resin exudation volume is associated with greater beetle resistance in ponderosa and other pines, other factors being equal (Smith 1975, Hodges et al. 1979, Smith 2000, Strom et al. 2002, Boyle et al. 2004, Wallin et al. 2008).

Techniques for measuring pine resin defenses are a topic of current and historic debate. Early studies focused on oleoresin exudation pressure (OEP), the pressure of the resin inside the canals (Vité 1961, Vité and Wood 1961, Mathre 1964). However, subsequent research has questioned the predictive value of OEP (Stark 1965, Lorio 1994, P. Lorio, personal communication 2001), and the method of choice in recent times has been to directly measure the volume or mass of oleoresin flow (sometimes termed oleoresin exudation flow, or OEF). Resin flow is typically assessed by funneling resin that emerges from drilled or cut bole wounds into test tubes over a certain period of time, such as 24 hours (e.g. Feeney et al. 1998, Strom et al. 2002, Wallin et al. 2003, Perrakis 2004, Wallin et al. 2008).

The physiology of resin production and storage are important factors in its defensive properties. In most pines, resin is produced and stored in pre-formed networks of sapwood and phloem canals (Büsgen and Münch 1929, Bannan 1936, Lin et al. 2002). The resin canals consist of an interconnected elongated network in which epithelial parenchyma cells form the canal walls. Resin is synthesized in epithelial cells and secreted into the intercellular space (the resin canal), where it is stored under considerable pressure (Büsgen and Münch 1929, Vité 1961). Resin pressure appears strongly and positively correlated with cellular turgor pressure and soil

moisture availability (Vité 1961, Hodges and Lorio 1971). When a duct is severed, such as during bark beetle attack, the internal pressure forces the resin out at the wound site. The epithelial cells expand and continue exerting pressure on the resin as it is depleted, collapsing the duct in the process. After the wound has sealed (via monoterpene volatization and crystallization of resin acids; see below), the epithelial cells slowly renew the resin supply, returning the ducts to their normal cylindrical shape in the process (Büsgen and Münch 1929, Fahn 1979).

Little information is available on the specific resin canal characteristics of ponderosa pine. Some of the older articles present their findings as pertinent to the entire genus, despite experimentation in only 1 or 2 species of *Pinus* (e.g. Büsgen and Münch 1929, Bannan 1936). More recent observations of large within-tree variation in resin flow (such as on different sides of a single tree) support the notion of resin canals being primarily interconnected in vertical and radial orientations, and absent in tangential directions (e.g. Lombardero et al. 2000, 2006, Perrakis 2004, D. Wood, personal communication 2001; C. Nelson, personal communication 2005).

Chemically, resin is composed of resin acids (diterpenes, C_{20} molecules) commonly known collectively as rosin, dissolved in a liquid mixture of monoterpenes (C_{10}) and sesquiterpenes (C_{15}). The latter two components constitute natural turpentine, which is an easily volatized solvent (Phillips and Croteau 1999, Seybold et al. 2000). Thus, when resin is exposed to air, the turpentine fraction evaporates, leaving the solid resin acids behind, resulting in the commonly observed drops of crystallized resin covering wound sites on physically damaged conifers. In ponderosa pine, the monoterpenes comprise about 25% of the total resin weight (Smith 2000).

The monoterpene fraction constitutes the volatile portion of oleoresin and has therefore been extensively studied in the context of beetle host selection. The main monoterpene compounds in *Pinus* resin are α-pinene, β-pinene, 3-carene, sabinene, myrcene, limonene, and β-phellandrene; however, differences in monoterpene composition exist between species, geographic locations, and even individual trees (Smith 2000), complicating research efforts. Monoterpenes are considered toxic to beetles (Byers 1995, Smith 2000) and other pathogens (Himejima et al. 1992, Hofstetter et al. 2005). Limonene is the resin constituent believed to be the most toxic to attacking western pine beetles, followed by δ-3-carene, myrcene, β-pinene and α-pinene (Smith 1975, 2000). However, several of the monoterpenes are also used by beetles as precursors for the synthesis of pheromone attractants, with various combinations being used by different beetle species for chemical signaling purposes (Seybold et al. 2000, 2006).

Sturgeon (1979) studied resin chemistry in ponderosa pines in northern California and southern Oregon, finding that limonene content in the resin of individual trees was correlated with historic local bark beetle presence. He further suggested that the beetles were exerting selection pressures on these trees for higher limonene content in their resin. This co-evolution theory was expanded by other researchers, with evidence that the development of ponderosa pine's elaborate resin defenses was countered by the beetles' development of attractant pheromones out of oleoresin components (Mitton and Sturgeon 1982, Seybold et al. 2000). Recent reviews of chemical ecology and resin biochemistry (Byers 1995, Seybold et al. 2000, 2006) have offered some generalized observations on beetle-host chemical evolution. In particular, they noted (1) the sophisticated yet relatively common scolytid adaptations to conifer defense chemicals that involves using them as chemical precursors to pheromone attractants, and

(2) the remarkable ability in beetles to occasionally produce the same compounds *de novo* – without actually any use of host tree compounds.

Fire adds an additional level of complexity to the picture, one that has received little attention until very recently. Fire can have various effects on trees, including direct effects on resin production, exudation, and storage. Chemical changes to resin composition may also ensue; these changes may be similar, or different from changes induced by other physical stresses.

Several studies have reported increases in the resin flow of pines following fire (Feeney et al. 1998, Ryan 2000, Santoro et al. 2001, Perrakis and Agee 2006, Knebel and Wentworth 2007), or, more generally, increased resin flow or resin canal density following physical injury or other types of acute stress (Bannan 1936, Fahn and Zamski 1970, Lewinsohn et al. 1991, Ruel et al. 1998, Phillips and Croteau 1999, Lin et al. 2002). Given the previous discussion, and the general consensus that resin flow is positively correlated with beetle resistance, this response seems counterintuitive and merits investigation. An increase in tree defenses following fire does not match with the frequently reported post-fire increases in beetle attacks and tree mortality. Investigating this disconnect proved to be a major premise of this study.

The Growth-Differentiation Balance (GDB) theory relates to how plants balance the allocation of photosynthates between cellular growth and secondary metabolic processes. Originally developed based on studies on herbs and crop plants (Loomis 1953, Herms and Mattson 1992), GDB principles are now supported, at least in part, by evidence from a considerable body of experimental work on conifers (e.g. Lorio 1986, Lorio et al. 1990, Herms and Mattson 1992, Johnson et al. 1997, Wilkens et al. 1998, Lombardero et al. 2000). In the evolution of carbon allocation theories, GDB is a recent contender, subsuming previous autecology theories on growth controls (Stamp 2003).

The main premise of GDB theory is as follows: photosynthesis produces carbohydrate energy that is stored in plant tissues or allocated directly to plant structures to based on source/sink relationships that vary by environmental conditions and phenology. Growth processes and cellular differentiation (specialization) are competing sinks for carbohydrates, but growth is usually prioritized over differentiation (Herms and Mattson 1992). Environmental conditions that constrain growth, however, tend to be less limiting to photosynthesis (Hsiao 1973, Luxmoore 1991). Thus, when resource availability and environmental conditions (e.g. water, nutrients, temperature) are optimum for a species of interest, growth processes of cell division and enlargement dominate the energy allocation budget. When sub-optimal environmental conditions occur without limiting photosynthesis (such as mild or moderate water stress, Hsiao 1973), growth is immediately reduced, but carbon assimilation can often continue. The corresponding buildup in available carbohydrate energy is then available for allocation to cell differentiation and secondary metabolism (Lorio 1986; Herms and Mattson 1992). The conceptual model is illustrated in Figure 1, demonstrating that at intermediate to high levels of resources, reductions in resource availability will reduce overall growth, but increase secondary metabolism. Since pine resin ducts and oleoresin are produced via cellular differentiation, the physiological reasoning suggests that conditions favoring differentiation will result in increased energy investment in plant defenses.

In this discussion, 'growth' refers generally to irreversible cell division and expansion, while 'differentiation' refers to the transformation of young cells into specialized forms, such as secretory tissues, defensive structures, vascular tissue, certain reproductive tissues, etc. (Loomis 1953, Herms and Mattson 1992, Taiz and Zeiger 1998). Both of these processes require.

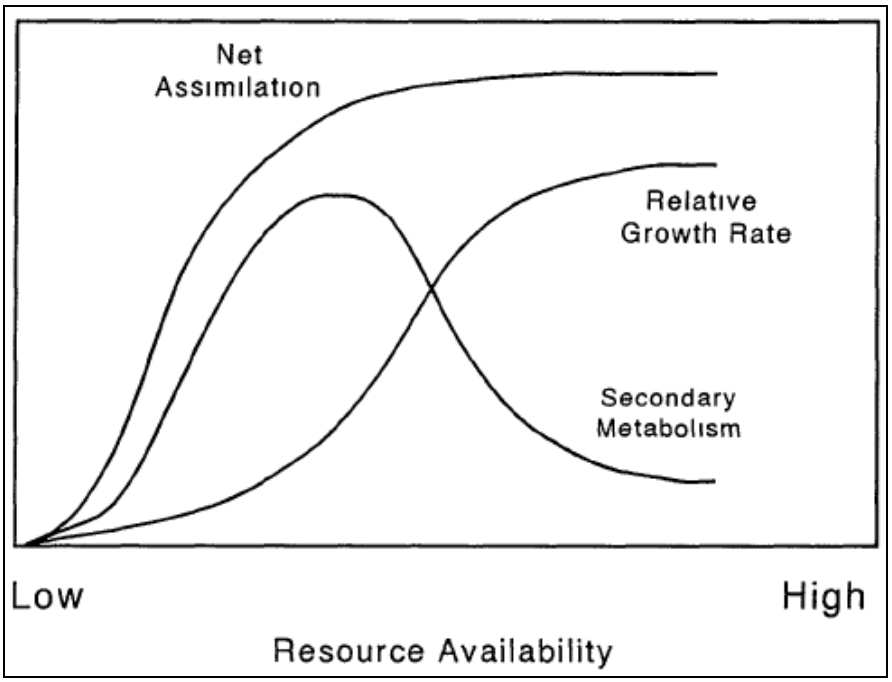

Figure 1. The theoretical relationships between resource availability and assimilation (photosynthesis), growth rate, and secondary metabolism (cell differentiation). From Herms and Mattson (1992).

carbohydrate energy inputs in the form of sucrose and other sugars (Kozlowski and Pallardy 1997a)

Carbohydrate allocation in trees is believed to be determined by source/sink relationships that vary by factors such as season, developmental stage, and in response to environmental stresses (Lorio 1993, Luxmoore et al. 1995, Kozlowski and Pallardy 1997a). These are the processes that determine where and when carbohydrates are allocated in the face of tradeoffs between competing physiological processes. In evergreen species in temperate climates, carbohydrates are produced year-round, and often accumulate during the winter, to be later translocated and depleted during the subsequent growing season (Kozlowski and Pallardy

1997b). Carbohydrates are transported vertically through the phloem from source tissues (leaves and shoots) to active sinks (meristems or sites of cellular activity, including epithelial cells), and laterally via the cambium and xylem rays (Luxmoore et al. 1995, Kozlowski and Pallardy 1997a). The ordering of priorities inevitably varies by species, genotype, season, development stage, and other factors, but in conifers, apical (shoot) and root meristems are high priority for carbohydrate allocation; cambial growth is somewhat lower priority, and defense is considered the overall lowest priority (Waring and Pitman 1985).

A key to the utility of GDB theory in the current context is the notion that certain levels of resource availability exist that will restrict growth, but still allow photosynthesis to continue (Herms and Mattson 1992). Specific resource levels will clearly be species- and site-dependent, but water (Johnson et al. 1997, Lombardero et al. 2000, McDowell et al. 2003) and nutrients (Wilkens et al. 1998) are typical candidates, where responses to varying resource levels have at least partly confirmed GDB predictions. Water deficit is the most frequent source of environmental stress in plants (Kramer 1983), is believed to be an important factor affecting tree survival at Crater Lake (Agee 2003) and was therefore a focus of investigation in this study.

In the context of pines and oleoresin, the formation of resin ducts requires highly specialized cells and structures (networks of epithelial cells), and therefore heavy carbohydrate investment in differentiation processes. Therefore, resin duct formation should be favored under suboptimal conditions, when growth is restricted but carbon assimilation can continue. This concept has been applied to conifers most extensively in studies on loblolly pine seasonality: early in the growing season, with abundant soil moisture, growth predominated over resin production; as water deficit increased later in summer, resin canal formation and resin flow increased sharply, in parallel with latewood formation (Lorio and Sommers 1986, Lorio et al.

1990, Lombardero et al. 2000). This response pattern is depicted in Figure 1. To date, research on conifer trees has shown mixed agreement with GDB theory – several studies support it, in broad terms (e.g. Lorio et al. 1990, Feeney et al. 1998, Wilkens et al. 1998, Lombardero et al. 2000). However, many potentially confounding factors can exist in field studies, and GDB theory certainly has not fully explained all the variation in the production of secondary metabolites (cf. Johnson et al. 1997).

Fire is important to these relationships because of its effects on tree carbon budgets and water uptake capacity, and perhaps also physical injury. Fire causes injury via bole charring, crown scorch, and root damage (Agee 1993). At Crater Lake, root losses appeared particularly important following prescribed fire, because of the slow combustion of thick duff layers and high root density in the duff (Swezy and Agee 1991), a process commonly observed in large, fire-excluded ponderosa pines (Ryan and Frandsen 1991). Crown scorch was also a factor on some burn sites and was positively correlated with bark beetle attacks (Swezy and Agee 1991).

Root losses from burning can obviously provoke water stress. Based on GDB principles, at some level of water deficit, growth of all tree structures is compromised while most photosynthesis continues. The loss of carbohydrate sinks from growing meristems would then be expected to increase allocation to cell differentiation, including production of resin canals. This could explain the increase in resin production that was observed in the season following burning at the Crater Lake sites (Perrakis 2004). In the long term, however, severe injury to the root system would unbalance the root/shoot ratio (Kozlowski and Pallardy 1997a), severely limiting water availability until roots were repaired (or foliage was dropped), perhaps over several seasons. Expected effects could include shortened growing seasons, ultimately reducing both

growth and photosynthesis, reduced tree vigor, and increased susceptibility to bark beetles and other pathogens.

Crown scorch has also been associated with successful beetle attacks in ponderosa pine at Crater Lake and elsewhere (Swezy and Agee 1991, Harrington 1993, McHugh and Kolb 2003). While effects of crown reductions on water relations are less obvious due to internal feedback effects (Ryan 2000, Wallin et al. 2003), losses of photosynthetic foliage necessarily reduce carbohydrate production, and should therefore result in decreased allocations to both growth and resin defenses, as observed by Wallin et al. (2003).

Such a pattern of dynamic response of tree defenses to environmental conditions and injury can potentially explain observed beetle-related overstory mortality at Crater Lake. In previous studies, the peak of successful beetle attacks occurred between 2 and 8 years following burning (Thomas and Agee 1986, Agee 2003). Continued monitoring of burned trees from the 2002 prescribed fires (Perrakis 2004) will help reveal the duration of resin flow response: based on GDB predictions, increased resin flow is expected to be temporary. Over several years, resin flow in injured trees should decrease relative to unburned controls, similar to the findings of Fredericksen et al. (1995), and consistent with observed bark beetle infestation patterns (Figure 2).

In typical wildfire and prescribed fire situations, the effects of root damage, bole charring and crown scorch occur together. However, their effects on physiological processes may be different in different regions of a tree, confounding efforts to understand whole-tree responses.

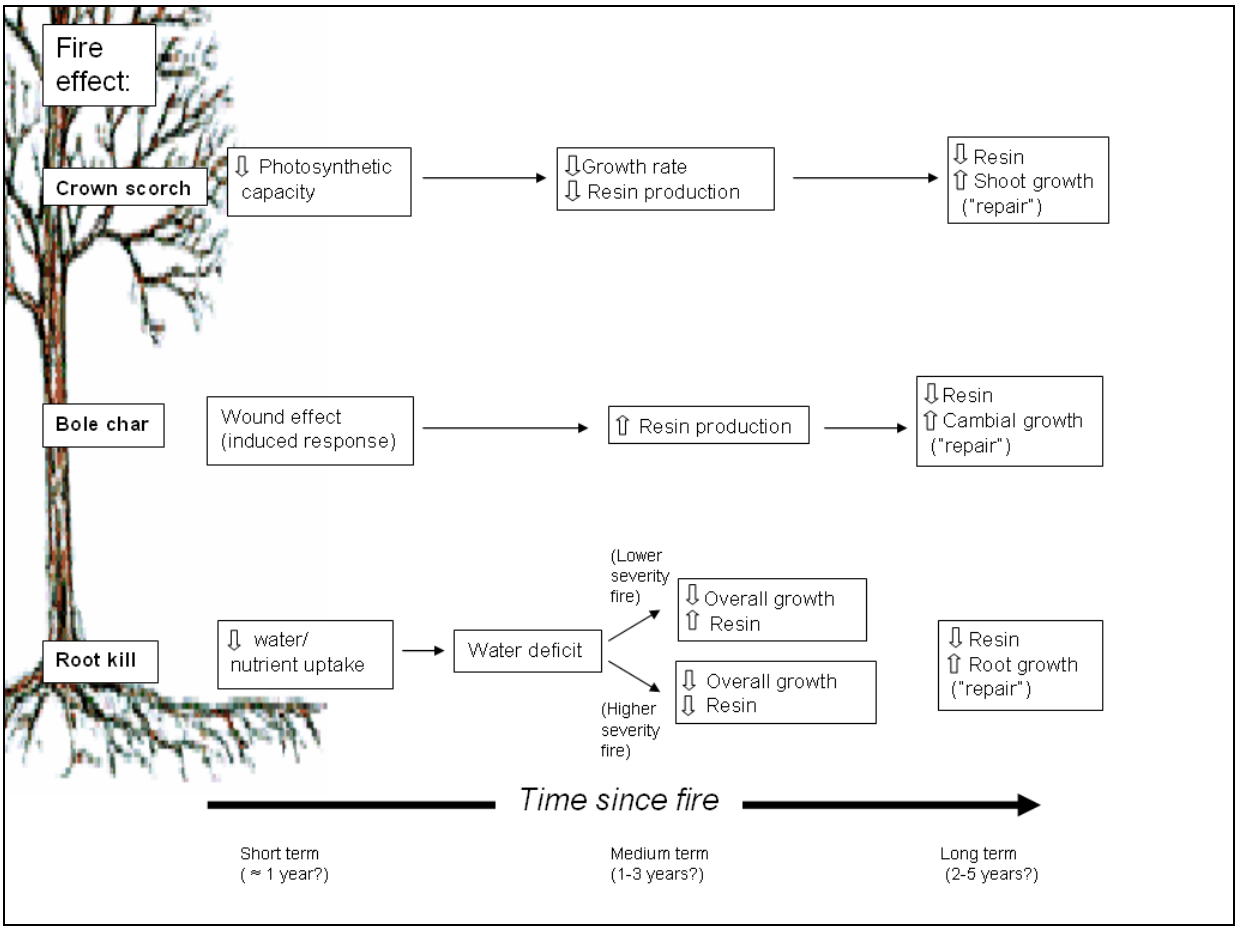

Figure 2. The hypothesized impacts of fire injury to a tree's crown, bole, and roots on growth and resin production, based on predictions of the Growth-Differentiation Balance theory.

Experimental Hypotheses

Based on the dynamic interactions described above between fire, host tree response, and beetles, the experiments in this study were designed to answer the following questions related specifically to the ecology of fire-bark beetle interactions in southern Oregon ponderosa pine forests:

- For how long is the elevated ponderosa pine mortality signal (Agee 2003, Perrakis 2004) detectable after spring and fall burning treatments in this ecosystem?
- What is the rate of fuel buildup after spring and fall prescribed fires?
- Can post-fire resin response in ponderosa pine be explained by the effects of injury to tree structures? That is, can root damage, crown scorch, or bole charring alone properly imitate observed fire effects on resin defenses?
- Are carbohydrate allocation tradeoffs between resin production (as measured by resin flow) and cambial growth detectable in ponderosa pines? How does fire injury affect these tradeoffs, and can they be explained by the Growth-Differentiation-Balance theory?
- What is the seasonal pattern of resin flow in ponderosa pines?
- Is the elevated post-fire resin response dependent on measurement height on the bole? Is elevated post-fire resin response a localized phenomenon?
- A previous study (Perrakis 2004) observed increased post-fire resin flow in ponderosa pines at Crater Lake NP. What is the duration of that response?
- Following fire and fire injury, do ponderosa pines produce different resin monoterpenes?

Methods and Results

The study was conducted primarily in the vicinity of Crater Lake National Park in southern Oregon (Figure 3). Continued monitoring of prescribed fires conducted in 2002 was done just north of the Panhandle area of the park. Ponderosa pines at this site were old-growth trees ranging in size from 40-200 cm diameter at breast height (dbh). Other studies were done at Sun Pass State Forest, where the ponderosa pine were pole-size (35-55 cm dbh), and on the Winema National Forest, just east of the panhandle. Limited studies were conducted on the Okanogan-Wenatchee National Forest in Washington State.

Six experiments were designed to test the experimental hypotheses. Methods and Results are summarized in the following order for each experiment:

Experiment 1. Vigor and survivorship of spring- and fall-burned trees, and fuel buildup after burning.

Experiment 2. Resin flow monitoring in spring- and fall-burned trees.

Experiment 3. Seasonal resin flow and fire-injury surrogates.

Experiment 4. Isolated bole charring and resin flow at different heights.

Experiment 5. Fire effects on resin chemistry

Experiment 6. Methods testing between resin sampling techniques

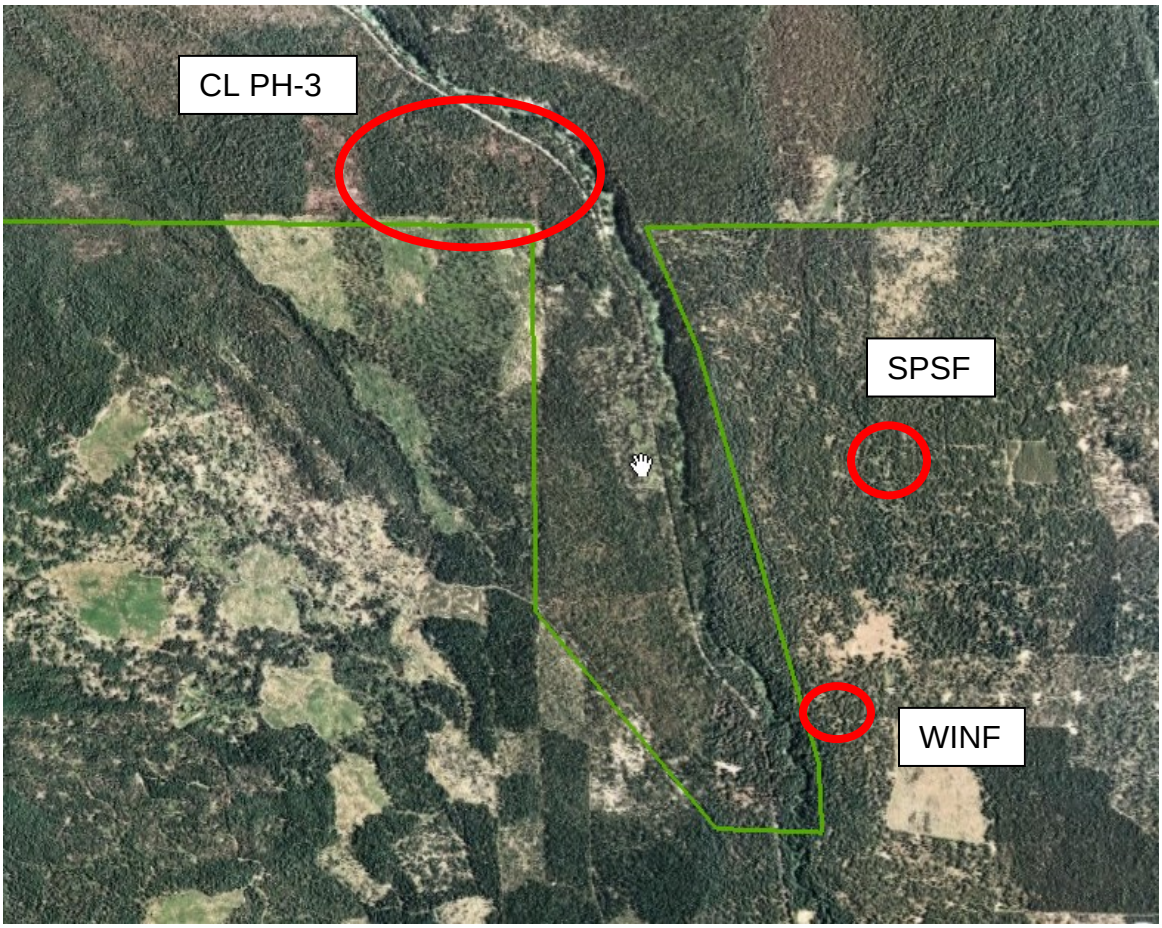

Figure 3. Overview of the southern boundary area of Crater Lake National Park, with the main study areas for several experiments shown highlighted. The park boundary is approximately represented by the green line. CL PH-3 represents the Crater Lake NP Panhandle-3 unit, where the 2002 prescribed burns were conducted (Experiments 1, 2); SPSF is the approximate location of the Sun Pass State Forest site (Experiments 3, 4), and WINF is the Winema National Forest site (Experiment 5). Most of the lower right section of the figure is contained in Sun Pass State Forest, but a small area (not shown) adjacent to the 'panhandle' formation is part of Winema National Forest.

Experiment 1. Vigor and survivorship of spring- and fall-burned trees, and fuel buildup after burning.

Vigor and survivorship. The issue of post-fire ponderosa pine mortality has been discussed at length in earlier sections, and this experiment was designed to follow up previous observations with some longer-term monitoring data. As previously described, prescribed fire was applied in spring and fall treatments to a ~70 ha area in the southern end of Crater Lake National Park, with known concerns related to immediate and extended ponderosa pine mortality (see Appendix 2, Perrakis and Agee 2006). The present experiment involved monitoring the survivorship and beetle presence in the old growth ponderosa pine population for 3 additional years following the 2003 monitoring and analysis session (which was summarized in Perrakis 2004). This longer term evaluation was intended to provide additional insights into the issue of extended post-fire pine mortality and the role of bark beetles (c.f. Kolb et al. 2007). In addition, ring-width data was collected from certain trees to test the correlation between radial growth indices and Keen's vigor class, which have previously been strongly associated with probability of mortality (Keen 1943, Swezy and Agee 1991, Perrakis 2004).

As described in Perrakis (2004), the prescribed fire treatments were conducted in spring and fall 2002. Diameter and crown vigor class were initially assessed on 1725 large ponderosa pines before treatment in 2001 and 2002, with crown vigor class being based on the 'California Pine Risk-Rating System', as described by F.P. Keen (1943). This method involves a qualitative assessment of overall tree vigor based on crown appearance and approximate age. The entire sample (1725 trees) was monitored annually for mortality, including an assignment of cause-of-death for those trees that died, during the 2002-2006 period. Although diameter at breast height data was collected from all trees, this factor was insignificant in predicting mortality in previous

analyses (Perrakis 2004), and so was not incorporated into the model. Likewise, 96 of the trees were subjected to resin flow sampling (see Experiment 2) and previous resin exudation pressure measurements (described in Perrakis 2004). These sampling efforts were deemed to have had negligible effects on the trees and were not factored into the mortality analysis.

Increment cores of the outer xylem rings were collected in summer 2005 from 10 trees per experimental unit. In each unit, cores were taken at breast height (1.4 m) from the north sides of 5 A- or B-class trees, and from 5 C- or D- class trees (Keen 1943); trees were randomly selected within each unit out of the pool of available A/B and C/D class trees, with only trees alive at the time of coring being selected. Since only the outer rings were analyzed, no effort was made to collect complete cores to the pith. Once collected, standard storage and mounting techniques were used. On all cores, Mahoney's Periodic Growth Ratio (PGR) and the number of rings in the outer centimeter were measured and computed. In previous studies, the PGR index has been found to be higher, and rings per cm lower, in beetle-resistant than in beetle-killed trees (Raffa and Berryman 1982, Stuart 1984).

This analysis used logistic regression on individual tree data (Dalgaard 2002). Treatment (spring burn, fall burn, or control) and Keen's crown class (class A, B, C, or D) were the categorical predictor variables. The model was fitted twice: once for the entire sample (N=1725), and once excluding trees that were believed to have died without evidence of insect attack (i.e. direct mortality from fire, windthrow or other causes; N=1701). The second run was designed to isolate the major factor of interest to this study, that being post-fire attacks by bark beetles and other insects or pathogens.

Increment core data was analyzed using a simple one-way simple analysis of variance. Mahoney's PGR and the rings per outer cm were both evaluated as a function of Keen's crown

vigor class. Post-hoc pairwise differences were evaluated using Tukey's HSD test (Zar 1999) at the $\alpha = 0.05$ level of significance.

By fall 2006, 139 large ponderosa pines had died, representing 8.1 % of the original population of 1725. Of those that died, 24 were estimated to have been killed directly by burning or windthrow in 2002 or early 2003. Thus, mortality for 115 trees out of 1701 (6.8 %) was at least partly related to post-treatment insect or pathogen attack. Of the 115, the majority were found in spring burn or fall burn treatment units (Table 1).

The main effects logistic regression model was significant at the $\alpha=0.05$ level when run with (1) all trees and (2) only live trees and those killed by secondary mortality. The burn treatment factor was significant, with spring burning increasing the probability of mortality, and fall burning increasing it further yet. The crown class factor was also significant, with class B trees more likely than controls to die during the study, and C and D classes each further increasing that probability. The overall mortality pattern is evident in Figure 4: probability of mortality was inversely proportional to crown class, and increased from controls (lowest probability) to spring burns and fall burns (highest probability).

Mortality in spring and fall burn units was clearly highest within the first year after burning, and then dropped off steadily in the following years. By the end of the study, 2.3 %, 6.1 %, and 16.4 % of trees in control, spring burn, and fall burn treatments, respectively had died.

The tree coring work resulted in 239 viable cores being collected from trees in all 24 units (10 per unit, minus one core that was lost during analysis). The number of cores from each crown class was 16, 97, 110, and 16 from classes A, B, C, and D, respectively – proportions very similar to the study area population as a whole.

Table 1. Mortality of large ponderosa pines by treatment and Keen's crown vigor class, 2003-2006, excluding direct mortality from fire and windthrow. SB and FB are spring and fall burn treatments, respectively.

Dead ponderosa pines - secondary mortality					
Treatment:	Control	SB	FB	Total dead	Total in study
Crown Class:					
A	0	1	5	6	182
B	3	11	31	45	756
C	7	15	23	45	664
D	3	5	11	19	123
Total dead	13	32	70	115	
Total in study	600	575	550		1725

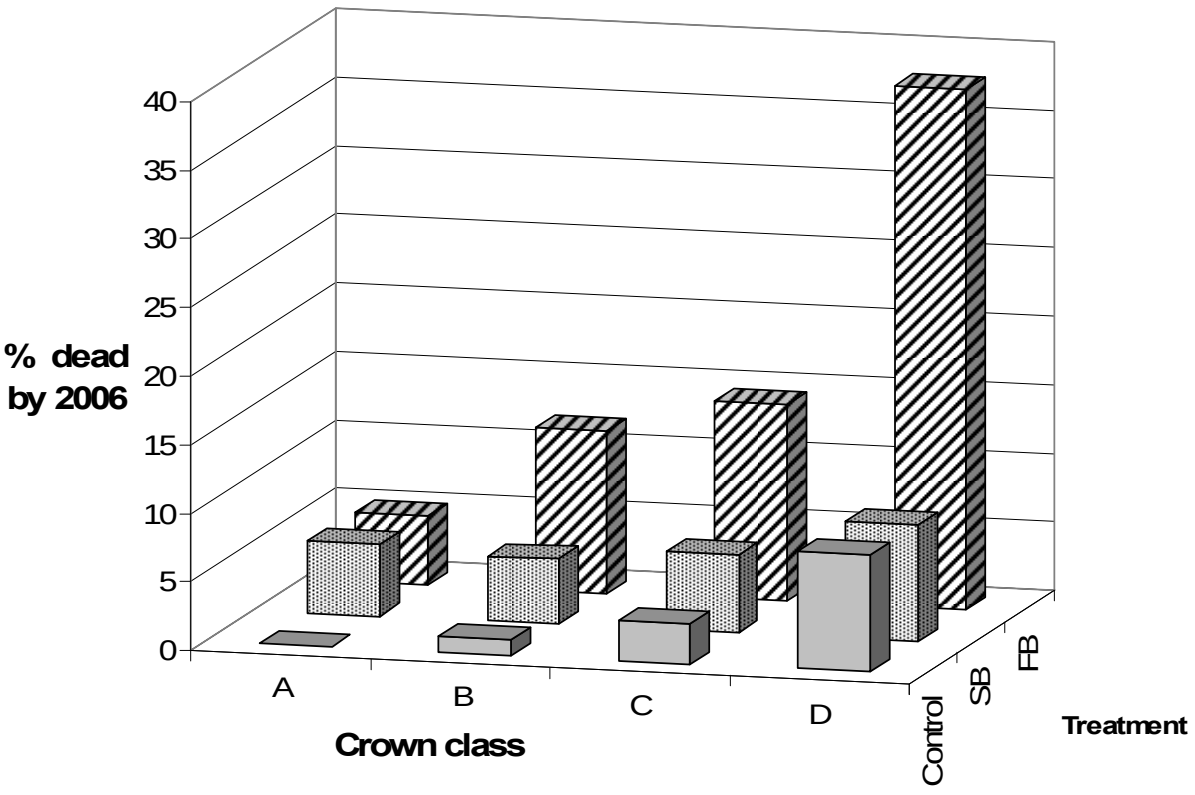

Figure 4. Post-treatment mortality of large ponderosa pines between spring 2003 and fall 2006, separated by Keen's (1943) class (A through D refer to crown vigor classes; see text) and burn treatment type (Control: unburned; SB: spring burn treatment; FB: fall burn treatment).

Mahoney's PGR was not significantly related to Keen's crown classes ($p = 0.471$). However, the relationship between number of rings per outer centimeter and Keen's crown class was highly significant ($p < 0.001$), with increasing number of rings/cm between classes A to D (along with high variability within groups; Figure 5). Differences between class A and B trees and between class C and D trees were not significant, but all other differences were significant at the $\alpha = 0.05$ level. Mean numbers of rings per outer cm (and standard deviations) for the 4 classes were A: 21.8 (11.9); B: 26.4 (13.2); C: 35.8 (15.3); D: 42.3 (17.4). The grand mean from all samples was 31.5 rings/cm.

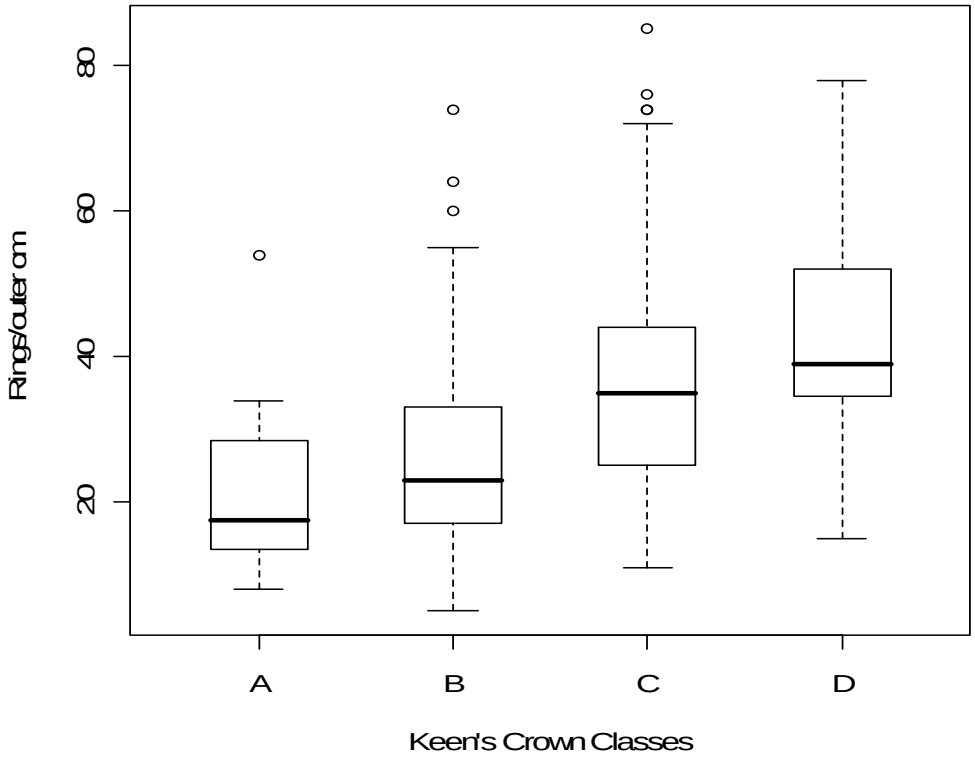

Figure 5. Boxplots of the number of rings per outer centimeter, grouped by Keen's (1943) crown classes. Center lines and boxes represent median and interquartile range values, respectively. Circles represent outlier values in each crown class. Numbers of tree cores analyzed in each class were 16, 97, 110, and 16, for classes A through D, respectively.

Fuel buildup. Fuels reduction is a major topic of interest in modern fire management, and prescribed burns are frequently-used treatment tools for modifying fuel loading in many types of forest ecosystems (Fernandes and Botelho 2003). This experiment consisted of an extension of a previous study on fuels changes following the previously-described spring and fall prescribed burns in 2002 in Crater Lake NP. The objective of this experiment was to monitor post-fire loading of dead woody fuels for two additional years following prescribed fire. Since the duration of treatment effectiveness of prescribed burning is uncertain and potentially short-lived, there is interest in tracking post-treatment changes to fuel loading over time.

Study area, prescribed burn treatments, and plot layout were described in Perrakis (2004). Surface fuels were measured along identical transects using standard methods on 3 occasions: in summer 2001/2002 (pre-treatment), in summer 2003 (1 year post-treatment), and in summer 2005 (3 years post-treatment). Standard planar-intersect methods were used (Brown 1974), with equation coefficients for appropriate species from van Wagtendonk et al. (1996). Litter and duff measurements were taken at 30 locations per experimental unit; fuel loading of these components was calculated from equations in Agee (1973). The total length of transect surveyed per treatment type (spring burn, fall burn, or control) was 1600 m (200 m per unit).

Data were analyzed using analysis of variance (ANOVA), followed by Tukey's HSD test for post-hoc comparisons (Zar 1999). Values were analyzed as the differences from pre-treatment values at the two post-treatment times: 2003 and 2005. Differences were compared separately between fine fuel classes (1, 10, 100 hr time-lag classes plus litter) and total fuel loading (all layers) at the α=0.05 level of significance. Results from all years are included for completeness.

As previously reported, fine and total fuel loading averaged 42.5 Mg/ha and 151.4 Mg/ha, respectively, before treatment, and differences between treatment groups were not significant. Analysis results are summarized in Figure 6. Fine fuel loading on control units showed slight fuel increases in both 2003 and 2005, suggesting some measurement variability or error between years. Both spring burns and fall burns had significant reductions in fine fuels in both 2003 and 2005 compared to controls; mean reductions were more limited in spring burn than fall burn units, but differences between burn seasons was not significant at the 0.05 level. Total fuel loading in control units indicated slight increases in 2003 and slight decreases in 2005.

Both spring and fall burning treatments also significantly reduced total fuel loading (fine fuel + coarse wood + duff) in both years. Mean reductions in fall burn units were greater than in spring burn units in both years, but differences between burn seasons were only significant in 2003.

Figure 7 shows the variation over time between the various components of the dead surface fuels. As the figure shows, the majority of the fuel mass was contained in the coarse woody fuels and forest floor components (litter and duff). In addition, variability in fuel loading in control units over the 3 measurement years suggests considerable measurement error or variability within experimental units. The most difficult and subjective fuelbed components to measure were the depths of litter and duff layers.

In terms of overall mean percentage change and recovery, by 2005, spring burn units had recovered 84% and 71% of pre-burn fine and total fuel levels, respectively; comparable figures for fall burns are 60% (fine) and 55% (total), while fuels measured on controls were 117% (fine) and 93% (total) of 2001 levels.

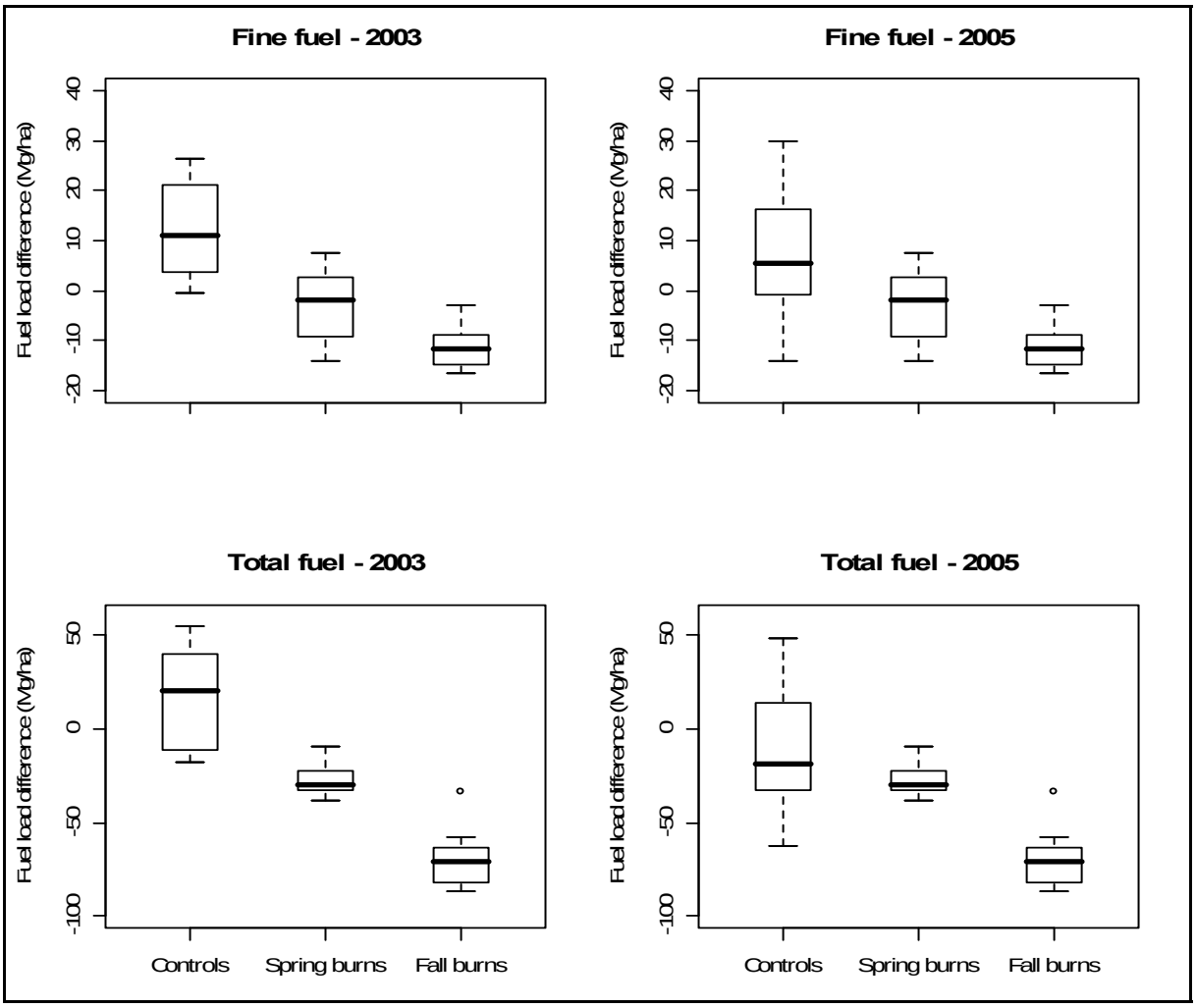

Figure 6. Boxplots showing variations in fuel loading changes between treatments at two post-treatment times. Pre-treatment measurements were conducted in 2001/2002 and data presented are differences from pre-treatment values. Center lines and boxes represent median and interquartile range values, respectively; circles depict extreme values.

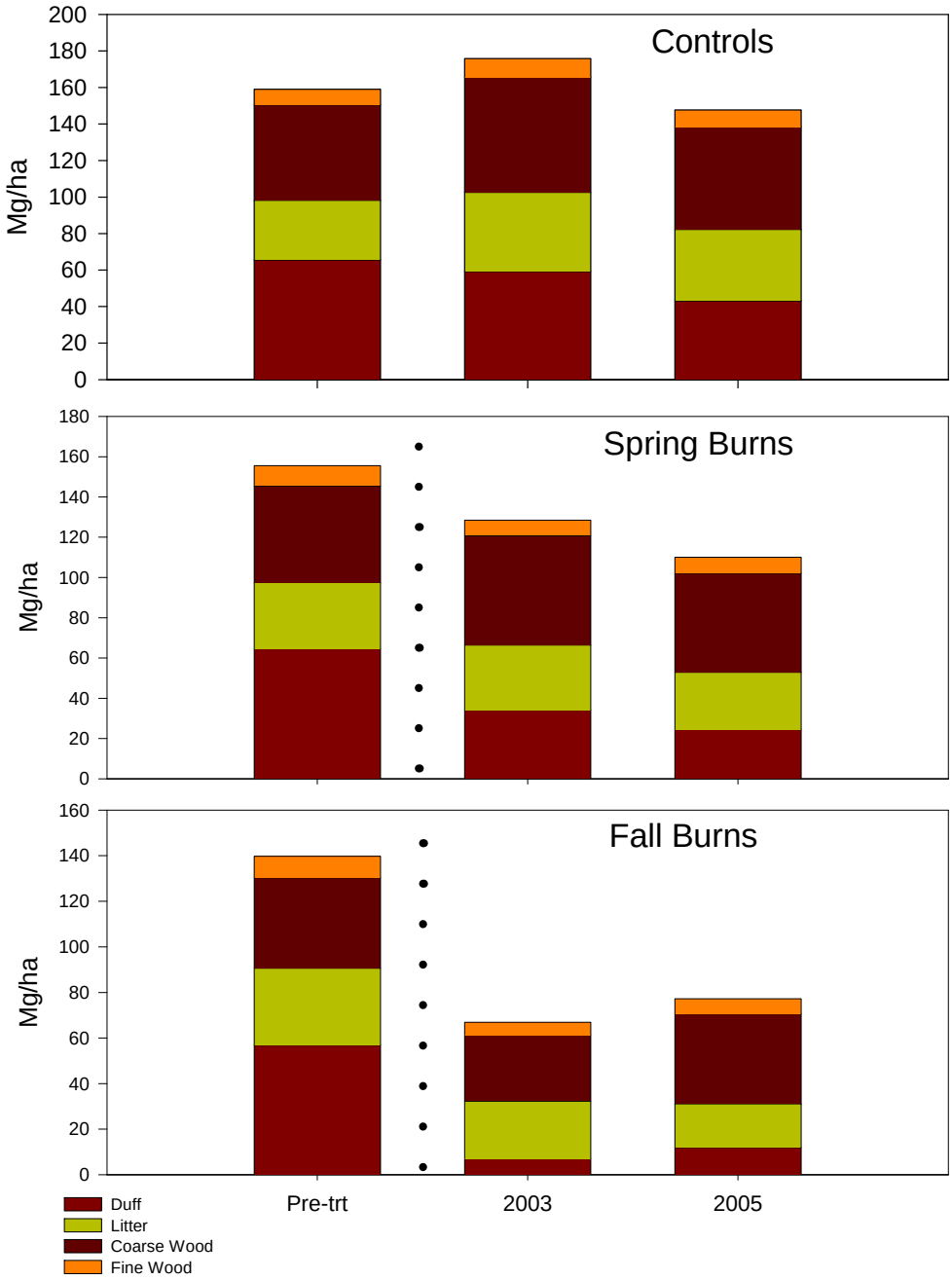

Figure 7. Average surface fuel loading at the three sampling times, broken down by fuel component. Dotted line represents timing of burn treatments.

The burn treatments successfully reduced both fine and total fuels, and differences were still statistically significant by 2005. However, the benefits in terms of reduced fuel loading were relatively limited, particularly in spring burn units, and likely short-lived as fire-killed biomass progressively becomes part of the surface fuels layer in the immediate post-fire years.

Experiment 2. Resin flow monitoring in spring- and fall-burned trees.

This experiment involved additional post-burn resin flow monitoring in large ponderosa pines from the Crater Lake prescribed burn site. As resin flow was previously found to increase following burn treatments (Perrakis 2004), the present experiment was designed to test the duration of this effect, as well as assess whether there was evidence that post-fire resin flow followed the energy allocation patterns predicted by growth differentiation balance theory. Spring burn, fall burn, and control treatments as well as high and low vigor (see Experiment 1) were contrasted for this purpose, with resin measurements taken for an additional 3 years beyond the previous (Perrakis 2004) dataset.

As previously described (Perrakis 2004), resin flow was measured on 96 large (40-170 cm DBH, average 90 cm) ponderosa pines before and after prescribed fire treatments, with 4 trees (2 high vigor, 2 low vigor) monitored in each of 24 experimental units (8 units each in spring burn, fall burn, control treatments). Half of the trees in each unit (1 high, 1 low vigor) were also subjected to a 'light raking' treatment prior to burning to evaluate the effects of this technique on resin flow. However, the raking treatment was not significant in any prior analyses on resin flow or pressure (Perrakis 2004) and is not further considered here. Trees were selected randomly for resin sampling based on Keen's crown vigor classes, the high vigor group consisting of A-class trees (or B-class, when no A-class trees existed) and the low vigor group consisting of C-class trees.

Resin sampling was performed using the methodology described in Perrakis (2004) (also see Figure 9 (left) in this report), with angled holes drilled into each tree bole at approximately breast height (1.2-1.5 m), brass funnels directing resin into centrifuge tubes, and resin volumes measured 24 hours after drilling. Two holes were drilled in each tree at each measurement time.

Because of logistical difficulties with the early OEF sampling, reliable pre-treatment resin flow data was collected only from the fall burn experimental units and half of control treatment units. As a result, the full dataset of resin flow values are compared only in terms of total resin volume at each time period; a separate analysis based on differences from pre-burn values was done only for the fall burn units. Pre-treatment sampling on fall treatments (burns and 4 control units) was conducted in early September, 2002. Measurements in 2003-2006 were conducted in mid-August of each year for consistency, with all measurements conducted within a few days of stable weather to avoid bias. Although 2002-2003 data was previously presented (Perrakis 2004), results from all years are included for completeness.

The structure of treatments and randomization at the scale of experimental units suggested a nested (split plot) design linear model (Oehlert 2000, Pinheiro and Bates 2000). Replication (the burn treatments) and tree grouping were at the level of experimental unit, with resin flow measured in the 4 sample trees per unit. As in the previous experiments in this study, resin volumes were square-root transformed with to stabilize variances. Categorical predictors included *Crown class* (high or low, based on A or C Keen's classes), treatment (*Control*, *Spring burn*, or *Fall burn*), and year (2003 – 2006).

The second analysis involved only the fall burns and 4 control units. For this model run, pre-treatment resin flow values were subtracted from each year's measured values (post - pre); differences could therefore result in either positive or negative values. Fixed factors (treatment, crown class, and year) and all interactions were initially included in the model; interaction terms were included and evaluated based on the $\alpha = 0.05$ level of significance.

Prior to burning, resin flow was not significantly different between trees in fall burn and control units (Perrakis 2004). In the first post-treatment year (2003), there were several instances

when sampling test tubes overflowed with resin in the highest flow samples. This was noted in 7 trees in fall burn treatments (4 high, 3 low vigor), 4 trees in spring burn treatments (3 high, 1 low vigor), and 1 tree in control treatments (high vigor). In following years, a switch to larger centrifuge tubes (50 mL) eliminated this problem. Thus, resin flow was slightly higher in 2003 than the values presented, particularly in fall and spring burn treatments.

None of the 2-way or 3-way interaction terms were significant and were dropped from subsequent model runs. Since the *Trt:Year* term was not significant ($p = 0.84$ in a full-factorial run), this suggests that mean treatment effects were not significantly different between the years considered in the study. All fixed-effect factors were significant at the 0.05 level. Both the *SB* and *FB* terms were significantly different from zero, suggesting that trees in both spring burn and fall burn treatments had significantly higher resin flow than in controls. Low vigor (*ClassC*) trees had significantly lower resin flow than high vigor trees. The overall *Year* effect is somewhat harder to interpret. Although the fixed factor term is significant, none of the coefficients for individual years are different from zero at the 0.05 level. Year-to-year variation in resin flow likely affected all trees, but these results do not suggest that they were a significant factor in any individual year during this experiment.

Trees in fall burn units had greater resin flow differences than those in control units, once again suggesting that resin flow increased post-fire. In this analysis, *Cclass* was not significant, suggesting that the trees' induced resin response was similar in the high and low crown vigor classes. The *Year* factor was also not significant in this analysis, indicating that relative resin flow change was constant across the study timeline.

The overall resin flow trend over the 5 years of the study (presented as means of all trees in each treatment group) is presented in Figure 8. The post-treatment response of increased resin

flow in burned trees is apparent from the figure, with both spring and fall burn treatments apparently peaking in the second year after burning (2004). By 2006, resin flow from spring burn and fall burn treatment groups has declined to levels nearly approaching control treatments once again.

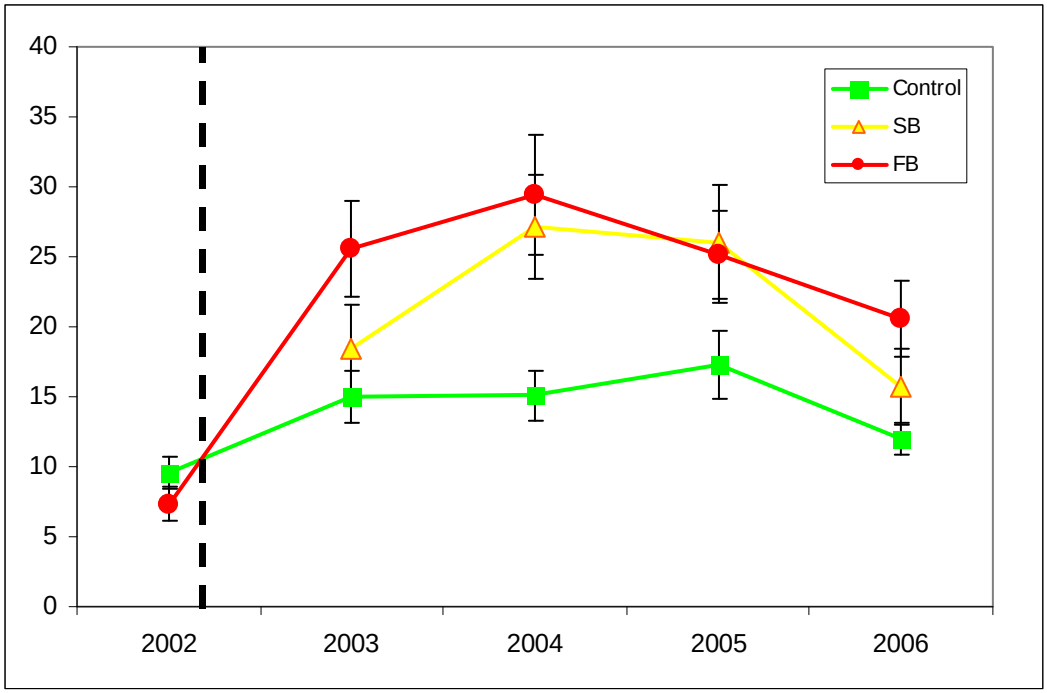

Figure 8. Untransformed resin flow means and standard errors (of unit means) by year and treatment group; n = 4 trees per unit.

Experiment 3. Seasonal resin flow and fire-injury surrogates.

This experiment related to the central premise of the study, that is, to examine the physiology of fire injury and resin defense response in pole-sized ponderosa pines. Based on defense physiology principles, the objective of this experiment was to subject trees to fire or various "fire-surrogate" treatments, and measure their resin flow response repeatedly over time. Various predictors would be used to assess the value of GDB theory in predicting post-fire energy allocations. Experimental treatments were designed to simulate fire injury to different tree structures (roots, crown), in turn affecting the trees' environmental resource budgets (light, water, nutrients), in accordance with the autecology principles described in the literature review – or so the theory suggested. Additional experimental objectives included assessing the seasonal pattern of resin response over several years and evaluating the sensitivity to injury (fire or mechanical) of pole-sized ponderosa pines. This experiment was carried on for 4 seasons, and involved considerable complexity and challenges in both implementation and analysis.

The experiment was conducted in a stand of pole-size ponderosa pines close to the Crater Lake NP 'panhandle' (Figure 3 - SPSF) and the site of previous fire-bark beetle investigations (Perrakis 2004). According Oregon Department of Forestry (ODF) personnel, the stand was pre-commercially thinned during the late 1980's, approximately 15 years previous to the start of the experiment. Chosen treatments included 2 levels of root disturbance (achieved by mechanical trenching) and one level of crown reduction (pruning) to simulate fire damage to these structures, in the sense of emulating root mortality from soil heating and crown mortality from scorching, respectively. These treatments were compared with a sample treated with prescribed fire, to compare fire-surrogate effects to actual fire effects, and with a control sample to reduce confounding due to background variability. To evaluate the effects of the treatments on the trees'

water balance, carbon-13 (^{13}C) discrimination was analyzed in xylem growth rings taken from increment cores. This technique will be discussed briefly. Naturally-occurring atmospheric carbon dioxide primarily contains ^{12}C, but also contains about 1.1 % of the ^{13}C isotope (Smith 1972a). Because of its heavier molecular weight, ^{13}C carbon dioxide is slower to diffuse across stomatal boundaries and less reactive to the photosynthetic catalyst Rubisco (ribulose 1,5 biphosphate carboxylase-oxygenase) (O'Leary 1981). Thus, plants discriminate against the heavier ^{13}C molecules during photosynthesis, and less of this type of carbon ultimately becomes incorporated into plant tissues. However, the degree of ^{13}C-isotope discrimination is related to stomatal conductance and ultimately, plant water balance: as water stress increases, so does stomatal closure; stomatal conductance decreases accordingly, and CO_2 remains trapped in photosynthetic mesophyll tissues. Thus, a greater proportion of ^{13}C becomes assimilated into plant tissues. ^{13}C-discrimination in xylem rings has therefore been positively correlated with soil water potential under water-limited growing conditions (McNulty and Swank 1995, McDowell et al. 2003); based on this mechamism, ^{13}C was used in this experiment as an indicator of overall tree moisture stress.

This experimental design was chosen to examine the pattern of ponderosa pine susceptibility to insect attacks over the course of several years after burning. Given the uncertainties regarding the timing of post-fire beetle outbreaks (Agee 2003, this study), it was deemed desirable to carry on the experiment for at least 3 years after treatments. Practical considerations placed an upper limit on the study duration, and in the end 4 years of data were collected, including the shortened initial season of treatment (2003).

The design of this experiment initially involved using high-resolution automatic dendrometers to track diurnal variations in stem diameter much more precisely than using

increment cores. Unfortunately, that aspect of the experiment was unsuccessful, as difficulties with the equipment resulted in the inability to obtain usable data. Ultimately, ring width data were used, providing a simple, if less precise (but perhaps more accurate) measure of annual radial growth.

Sample trees were split into 5 treatment groups: one group was subjected to a pruning treatment designed to remove the lower half of each tree's crown. Pruning was done by hand, removing live branches until a predetermined point representing half the crown volume was reached. A second group was subjected to mechanical root damage by trenching to a depth of approximately 50-60 cm, at a distance of approximately 1.5 m from the bole. Trenches were dug in three separate segments around each tree to add up to an estimated 1/3 of the tree's circumference ("light trenching"). A third group was subjected to the same trenching treatment, but dug around 2/3 of the each tree's circumference ("heavy trenching"). The fourth treatment group was subjected to a mid-summer prescribed burn. The 5 remaining sample trees were untreated controls. The burn treatment was assigned to a group of trees clustered in a ~0.2 ha area, based on operational requirements related to the prescribed burn; other treatments were assigned to trees randomly. Pruning and trenching treatments were conducted on June 26-27 and July 2-4, 2003, respectively. The prescribed burn treatment was conducted on August 2, 2003, with isolated smoldering until August 4. Prior to ignition (in mid-July), surface litter fuels were aerated using hand tools to reduce the duration of active fire for fire control purposes.

Resin flow was measured on all trees twice per month from mid-spring to early fall between 2003 and 2006. Measurements were timed as follows: 2003 (7 repeats): June 22 to Sept. 25; 2004 (8 repeats): May 16 (only 1 measurement in May) to Sept. 13; 2005 (8 repeats): May 27 to Sept. 14; 2006 (7 repeats): June 13 to Sept. 19. The 12-14 day delay between sampling periods

was chosen as a compromise between the benefits of frequent sampling and the drawbacks of causing frequent injuries (albeit minor) to sample trees. The period between sampling sessions was roughly estimated to be a minimum time delay for resin ducts to recharge (Büsgen and Münch 1929). Resin flow measurements were conducted as described in Perrakis (2004). Briefly, at each resin sampling time, 2 holes of 5.56 mm (7/32 inch) diameter were drilled on opposite sides of the lower bole region of the trees. Holes were drilled at an angle of approximately 30 degrees below horizontal and to a depth of 2.5 cm (1 inch, estimated) into the sapwood. A funnel "scoop" consisting of 6.35 mm (1/4 inch) diameter brass tubing, filed down on one side (see Figure 9, left method) was inserted into each hole to collect the resin and direct it into a 50 mL test tube.

Figure 9. Example of measuring resin flow using two different methods on one tree at Leavenworth, WA. The scoop method is on the left, and the exposed xylem of the arch-punch scar is visible on the right.

Resin yield in test tubes was measured after 24 hours. In cases where resin was obviously leaking around the outside of a scoop (rather than flowing into the test tube), 0.5 mL was added to the test tube value. After each sampling period, tubes were removed and small sections of clean hardwood dowel were hammered into the holes to end the resin outflow.

This method for measuring OEF was selected because of the small area of injury it creates for each resin sample (compare methods in Figure 9), and the ease of blocking the sampling holes following measurement. Over the course of the experiment, 60 holes were drilled in the lower bole region of each tree, roughly between about 0.8 m and 1.5 m height.

The variations in treatment timing (see above) resulted in differences between treatment groups in the numbers of pre-treatment and post-treatment resin flow measurements collected. Thus, out of the 7 resin flow measurements in 2003, only the first was a pre-treatment measurement in the pruned tree group (the other 6 were conducted at various times post-treatment); in the two trenched tree groups, 2 sets of resin flow measurements were pre-treatment measures; in the burned tree group, 3 of these were pre-treatment measurements. The variable timing of treatments and resin sampling had serious implications for data analysis (see below).

Annual radial growth data used in modeling resin flow were calculated from increment core xylem rings. Standard increment boring techniques were used to obtain outer sapwood cores for all trees in early spring 2007. Cores were taken on the northeast, north, or northwest sides of each tree at approximately breast height (1.37 m) and stored in plastic straws for protection and transport. At least three cores were taken from each tree; two cores were mounted, sanded, and used for measuring radial growth and one was dissected for carbon isotope discrimination (see below). Growth ring widths were measured manually using a digitally-projected light microscope with a movable stage. Basal area increment (BAI) was calculated as the difference

between successive basal area values (starting with 2003 DBH), using ring width values to increase each tree's radius every year. Average 1998-2002 BAI was also calculated for all trees. Foliar mass was estimated based on equations from Gholz (1982), which were derived from studies on northern Arizona ponderosa pines between 15 and 80 cm DBH. Based on visual estimates, the pruning treatment reduced crown volume by 50% in those trees; accordingly, foliar mass values were divided by 2 to obtain post-treatment values for these trees.

Carbon isotope discrimination was analyzed based on growth ring xylem in order to evaluate treatment effects on water balance. As mentioned above, cores were collected in early 2007 using standard techniques, and core handling and contact were minimized to avoid contamination. Annual rings were separated by hand using a sharp knife and packaged individually in sterile centrifuge tubes for transport to the laboratory. Carbon isotope analysis was conducted at the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, Flagstaff, AZ). Samples were processed using a Thermo-Finnigan Delta-Plus Advantage gas isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA) interfaced with a Costech Analytical ECS4010 elemental analyzer (Costech Analytical Technologies, Valencia, CA). $\delta^{13}C$, the difference in carbon-13 per mille between laboratory standards and experimental samples, was the value used in analyses.

Because of the complexity of this dataset, several different analysis approaches were used. Ultimately, resin volume was modeled as a function of several categorical and continuous predictor variables, including treatment, season, year, basal area increment, $\delta^{13}C$, and modeled foliar mass. Raw resin volumes were square-root transformed in order to stabilize variances and meet the assumptions of linear modeling. The 'season' predictor was a categorical variable based on sampling time, designed to separate the mid-summer resin flow peak (Harper and Wyman

1936, Smith 2000) from earlier- and later-season periods of lower flow. The variable was designed as follows: Season 1 (*s1*): May and June; *s2*: July to late August; *s3*: August 30 through September. Resin flow was measured once in all trees prior to treatment (22 June, 2003), and was initially modeled using the above predictors (except treatment, season, and year) to ensure there was no bias between treatment groups (Analysis 1). This was done using a simple linear regression model, with transformed resin volume as the response variable and treatment (*Trt*), average BAI (*avBAI*), $\delta^{13}C$, and *Foliar mass* as predictors:

Post-treatment resin volume was initially analyzed in terms of mid-summer average values in all treatments across all years (Analysis 2), to compare this dataset to the findings in the older Crater Lake trees (see Experiment 1 and 2). This was done by averaging the resin flow volume by tree in early and mid-August. Values in 2003 therefore represent the transformed average of resin volumes measured 1 to 1.5 months following trenching and pruning treatments, and 3 days and 2 weeks following the burn treatment. Measurement dates included in this first analysis were as follows – 2003: 05 and 18 Aug.; 2004: 02 and 15 Aug.; 2005: 01 and 16 Aug.; 2006: 01 and 21 Aug. Resin volume was modeled using a mixed-effects multiple linear regression model, initially as a full factorial model using *Treatment* and *Year* categorical predictors. Data were grouped by the random effect *Tree*, since resin measurements were not taken randomly, but rather repeatedly on the same sample trees (Pinheiro and Bates 2000). The model-fitting process included several iterations, as described in the Results.

Analysis on the larger dataset (Analysis 3) across all seasons, which included measurements from May to September, was done on 2004-2006 data, 1-3 years post-treatment. Data from 2003 was excluded from this analysis to avoid model-fitting errors due to nonexistent early 2003 post-treatment data. Model-fitting proceeded as before, with several continuous

predictor variables added to the previous model. Factors were successively eliminated based on the $\alpha = 0.05$ level or significance.

A final analysis (Analysis 4) was done on the differences from pre-treatment data. This was a simpler linear mixed-effects model that focusing on the burned trees, taking advantage of the three pre-burn resin measurements that were taken on this treatment group (22 June, 02 July, and 17 July 2003). The response values for the model were calculated for burned and control trees as the post- minus pre-treatment differences (untransformed) from mid-June, late June/early July, and mid-July for 2004, 2005 and 2006. Predictors consisted of treatment (burn or control), year, and the interaction between the two.

Other relationships in the data were also investigated. These included correlations between treatment and $\delta^{13}C$, to infer whether fire or mechanical injury treatments had detectable effects on whole-tree moisture stress. All analyses were performed using R version 2.7 (R-Project Collective 2008), and differences were evaluated at the $\alpha = 0.05$ level of significance.

Xylem ring width among all sample groups at the start of the experiment (1997-2002) was 2.8 ±0.60 mm (mean ± standard deviation), or 3.6 ± 0.86 rings/cm. Before any treatments were applied, resin flow was not significantly different between treatment groups. In addition, there was no evidence that average BAI (*BAI_av*), $\delta^{13}C$, or *Foliar mass* were significantly related to mid-June resin flow volume. Changing the order of the model terms also failed to reveal any significant probability values for any predictors.

Results from the first post-treatment analysis (Analysis 2, concerning August values, 2003 – 2006) showed that the treatment main effect factor (*Trt*) was not significant, but the *Year* factor and the *Trt:Year* interaction factor were both highly significant. The model summary contains one point of significant interest: immediately after burning, burned trees had

significantly lower resin flow than controls (*TrtF* variable). This was followed in the subsequent 3 years by significant resin increases in the burned group compared to 2003 (*TrtF:Year2004*, *TrtF:Year2005*, and *TrtF:Year2006* variables), particularly in 2004; however, with the terms in this order, the model does not establish whether this increase results in significantly higher values than in control treatment trees for the last 3 years, merely that resin flow was significantly higher under these treatment-year combinations than was accounted for by default treatment and year effects (control treatment and 2003 year). In addition, the *Year2006* variable was significant, suggesting that overall resin flows were lower in that year than in previous years. None of the other terms were significant in the model, suggesting that neither pruning nor the trenching treatments affected resin flow measurably.

To further clarify these results, the data (still August averages, 2003-2006) were analyzed with a slightly simpler model structure, this time with only the *Year* and *Trt:Year* predictor terms (since the main treatment effect, *Trt*, was non-significant in the first iteration). The findings are essentially the same as the full factorial model, with one key difference: in this form, the *Year2004:TrtF* variable was non-significant. Although the burned trees still had reduced resin flow immediately after burning in 2003, subsequent increases were too variable to be significant in the model. The *Year2006* term remained significant, showing an overall decrease in resin flow that year, as in the previous analysis.

The full dataset included measurements from early snowmelt to late summer. Figure 10 shows the resin volume values for control trees over the duration of the experiment – presented as a baseline of variability in untreated trees. The seasonal pattern is apparent, with one or two modal peaks in mid-summer, and much lower values early in the spring and early autumn.

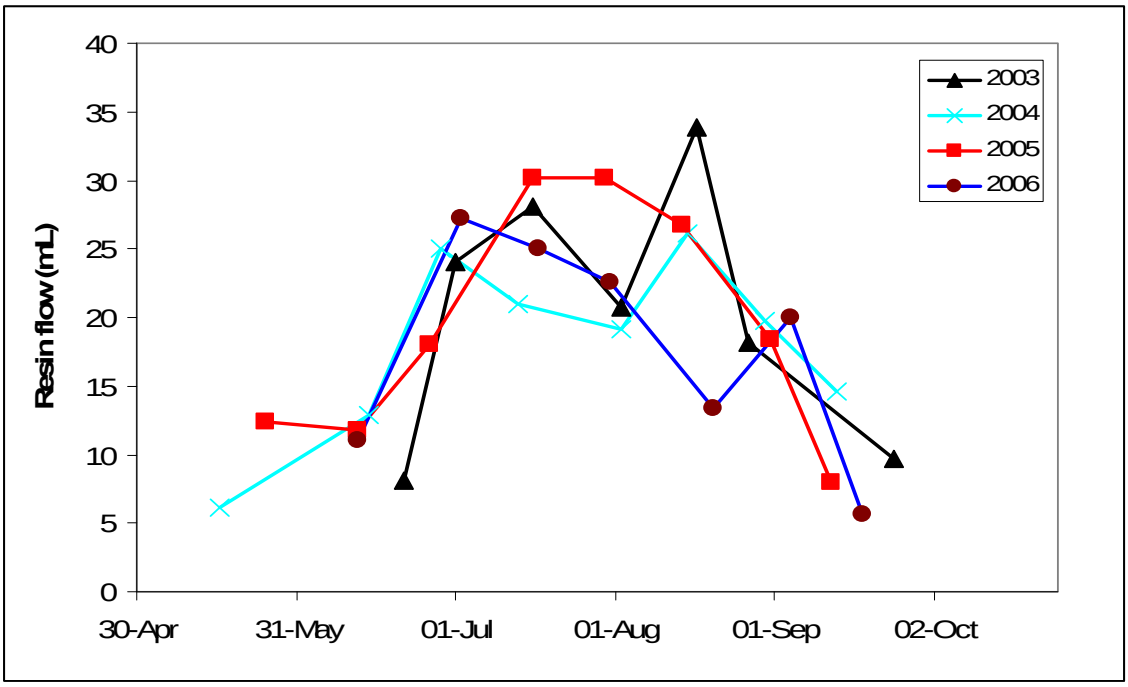

Figure 10. Resin flow for the control trees, 2003-2006.

Analysis of the full seasonal dataset included all 2004-2006 resin measurements (post-treatment years) from all treatment groups. The range of values produced is summarized in Figure 11. Several points are immediately apparent: if the control group (group 'C' in Figure 11) is assumed to be the default, it is clear that the frequency distributions of resin volume in most other treatments and trees are very similar. Only the burning treatment group (group 'F') stands out, with two trees apparently producing much higher resin volumes at certain times; one tree in the pruning group also stands out with somewhat higher resin flow. Focusing on the burning group, the two high resin flow trees are accompanied by two trees with average resin yields, and one tree with the lowest resin flow of the entire experiment. This outlier has a maximum resin volume that is lower than the median resin flow of nearly every other tree in the experiment. Before burning, the tree produced yields of 0, 0.5, and 0 mL of resin in early 2003, the lowest

values of the entire sample. After burning and in subsequent years, the tree yield up to 8 mL of resin, still much lower than the maximum yield of the next-lowest tree (17 mL in a control tree).

The model-building process quickly eliminated most predictors as non-significant. The only continuous covariate that looked significant was $\delta^{13}C$ ('c13.current'). The main effects factors Season and Year were highly significant at the 0.05 level, as was as the Season:Year interaction factor. The Trt:Year factor was nearly significant ($p = 0.052$). All other variables and interactions were eliminated from further model iterations.

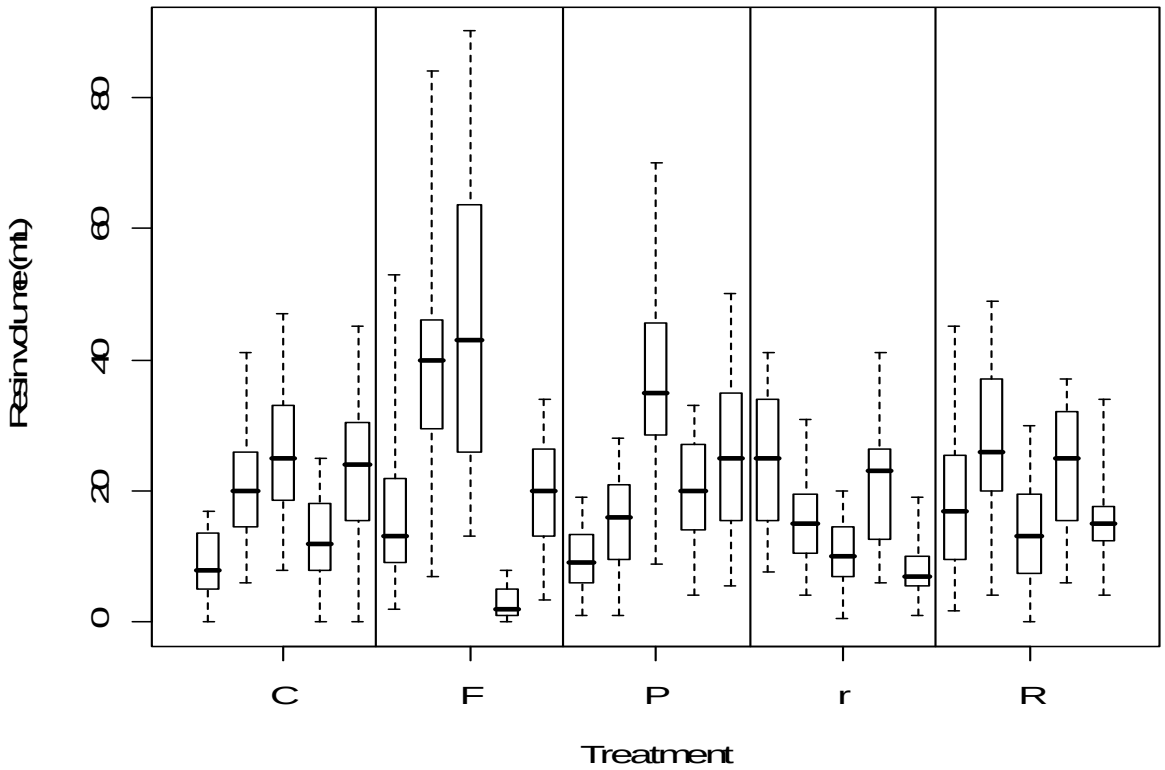

Figure 11. Medians, interquartiles, and ranges of resin volume by tree and treatment for all post-treatment data. C, F, P, r, and R refer to Control, Fire, Pruning, root trenching (light) and Root trenching (heavy), respectively.

For the second run, only $\delta^{13}C$, *Treatment*, *Season*, and *Year* and the 2-way interaction terms between categorical variables were included. Although $\delta^{13}C$ ('c13.current') showed a significant difference, the coefficient estimate was not significantly different from 0 ($\hat{\beta} = -0.049, p = 0.779$). That term was then excluded from the analysis. The *Treatment* main effect factor and *Trt:Season* interaction term were also excluded as non-significant at this point.

By the third iteration, the model contained little of analytical interest. Even the Year:Treatment interaction, the last term containing an experimentally manipulated variable, was not significant at the 0.05 level. This last model shows that transformed resin volume was affected by measurement season and year, and potentially their interaction.

The final analysis of these data (Analysis 4) was performed on the early season differences in the burned and control treatment groups. The intercept coefficient was not significantly different from zero, suggesting that control trees' resin volumes did not vary significantly from pre-burn values in 2003. Non-significant probability values for the year2005 and year2006 variables confirm that subsequent years' values for controls were also not significantly different from zero. Only the treatment effect was significant, and this time the interpretation is quite clear: burned trees had significant resin increases after treatment compared with unburned controls. Interaction variables showing differences among burned trees in subsequent years (a further increase in 2005 and a decrease in 2006) were not significantly different from zero, suggesting that the overall increase in resin volume remained relatively constant across all years.

Experiment 4: Isolated bole charring and resin flow at different heights

Following the first 2 seasons of monitoring in Experiment 3, it had become apparent that some of the prescribed burn samples were exuding much more resin than the fire-surrogate treatments (pruning or either of the trenching treatments; see Experiment 3). This fourth experiment was then designed to further test the possibility that bole heating and cambial injury were causing the observed resin increases. This required the development of a bole charring treatment that avoided any crown scorch or root damage effects. An additional objective involved evaluating resin response at different heights above the bole char site. Resin responses were measured before and after charring, on treated trees and controls at three heights on each sample tree, in order to reduce individual tree variability and test the vertical extent of the hypothesized response.

A pilot study was first conducted in October 2005 near Entiat, WA to refine treatment methods. The main experiment was held in Sun Pass State Forest, OR, adjacent to the Experiment 3 site. Pre-treatment sampling and the bole heating treatment were conducted in August 2006 and post-treatment data were collected one month (September 2006) and one year (August 2007) afterwards.

Twenty two high vigor trees were selected with diameters (DBH) between 30 and 55 cm. Half of these were randomly assigned to the bole heating treatment, while the other half were untreated controls. Prior to sampling and treatment, dead branches in the lower bole area were removed to allow safe access to the bole for attaching the resin collection apparatus. Disruption of live crown branches was minimal on all trees.

Resin flow was measured in all trees at three heights: 1.0 m, 2.75 m, and 4.5 m (±0.2 m), with two measurements (on approximately opposite sides of the bole) at each height level. Resin sampling for this experiment was performed according to Lorio et al.'s (1990) methods, using the resin samplers engineered and described by Karsky et al. (2004). Briefly, at each measurement location, tree bark was shaved and smoothed using a Log Wizard planing tool (Spruce Grove, Alberta, Canada; Figure 12A) attached to a small chainsaw body with a de-toothed chain. Within the smoothed area, a circular disc of the remaining bark and phloem was removed using a 1.27 cm diameter Osborne arch punch tool (Campbell Bosworth Equipment, TX), which leaves a circular scar of cut bark and phloem tissues but does not damage xylem tissue (Figure 12B). This OEF sampling method has been suggested to be more representative than other methods of resin defenses encountered by an attacking bark beetle, since it does not cause injury to the xylem tissues (Lorio et al. 1990). The exuded resin was captured by enclosed oleoresin sampler funnels (Karsky et al. 2004) directing resin into 50 mL centrifuge tubes. Samplers and attached centrifuge tubes were placed over the exposed xylem and screwed into place, forming a tight seal (Figure 12C).

Subsequent sampling locations at the three heights were offset vertically and horizontally from previous sampling wounds in order to minimize the potential effects of previous sampling in the same vicinity on the outer bole. At each measurement date, tubes were revisited twice to record total resin volume: 1 and 2 days after phloem removal (24 hours and 42 or 48 hours).

The bole charring treatment was conducted on August 6, 2006, following the pre-treatment resin sampling. Small platforms (~ 1m^2) were erected around the 11 treatment trees at a height of approximately 0.4 m, using wire fencing and commercially available tile backing board covered in metal foil (Figure 13). Charcoal briquettes were piled on these platforms

against the boles of sample trees on two opposing sides, in order to isolate heating effects to specific areas of boles; treated trees were thus heated along two quarters of their respective

Figure 12. Resin sampling. In clockwise order: A-Use of the Log Wizard planing tool; B- a close-up of a sampling location (photo was taken after resin sampling was completed; visible resin is dripping from sampler screw-holes on either side of the exposed xylem scar, as well as from the scar itself) C: a resin sampler and centrifuge tube installed on a tree, containing a small quantity of resin.

Figure 13. Bole charring treatment photos. Clockwise: A-Platforms and charcoal briquettes assembled and ignited; B-Close-up of burning briquettes; C-Position of briquettes around bole after combustion; D-Close-up of the burn scar on one side of a treated tree.

circumferences. Quantities of charcoal were estimated to be roughly equal per unit of tree circumference. Sections of damp canvas cloth were wrapped around tree boles beneath the platforms to catch any burning briquettes that might fall through the platforms to the ground. Briquettes were ignited and left to burn for approximately 1-1.5 hours following ignition; at that point, any remaining coals were scraped away. The resulting fire scar was clearly visible, extending through the bark and into the sapwood xylem (Figure 13-D).

In September 2007 it was noted that many of the previous year's sampling scars had been attacked by red turpentine beetles. The number of attacks was counted at each height level for further analysis to determine differences by treatment and height level.

Resin flow data were analyzed using linear mixed-effects models with a split-plot structure. Several versions of the model were fitted: the first iteration used pre-treatment resin volume as the response variable in order to test for inherent (e.g. genetic, site) differences between individuals in the two treatment groups. The second iteration tested the differences from pre-treatment values in a repeated measures design (comparing the two post-treatment measurement times) to test the effects of the charring treatment and variations in sampling height over two time periods.

Thus, the models were initially constructed with resin flow *Volume* (24 hour total from both sides of the tree at each height level) or volume difference (*Voldif*) fitted using treatment (*Trt*, 2 levels: control, burn) and *Height* (fitted as continuous variable) as the main fixed factors. Volume was square-root transformed, as before, to stabilize sample variances. The post-treatment model for *Voldif* also included the *Time* factor, set as a fixed-effect categorical variable since the two post-treatment measurement times were chosen deliberately and with no expectation of a unidirectional response over time (2 levels: 1 month and 1 year post-treatment).

Of greater interest was the *Trt* x *Time* interaction, which would assess whether the two treatments (char and control) had similar effects at the two time periods. Since multiple measurements were made on individual trees (measurements at different heights were not independent), the random factor *Tree* was needed to represent the nested structure.

Volpre and *Voldif* represent pre-treatment resin volume, and the post-treatment minus pre-treatment resin volume difference, respectively. *Trt* and *Time* are the categorical predictors described above, *Height* is a continuous covariate, and *e* represents the error term. Final models were chosen based on significance of the terms, model simplicity, and lowest Akaike's Information Criterion (AIC) values (Pinheiro and Bates 2000). Differences were evaluated at the $\alpha = 0.05$ level of significance.

As noted above, by September 2007, many sampling scars from the previous year had been attacked by red turpentine beetles, as evidenced by their large and unmistakable pitch tubes. However, the beetles attacked the majority of scars irrespective of height or treatment. Because of the potentially confounding effects of having intermixed charred trees and controls in close proximity (as well as very similar mean attack proportions, irrespective of treatment or height level), no further analysis of these data was undertaken.

In terms of resin flow, high variability was encountered between different trees and measurements, as expected from previous trials and experiments on oleoresin flow. The sampling methodology was also not free of problems, with several instances of observed resin leakage between the bark-sampler interface, as well as a few cases where resin was suspected to have drained into bark crevices at the bottom of the punched scar area instead of draining into the resin sampler. Instances where leaking was identified had 0.5 mL added to their resin volume quantities.

Initial model fitting was attempted as described above. In total, 396 samples were taken (22 trees, 3 time periods, 3 height levels, 2 sides measured per height level); measurements on opposite tree sides at each height level were totaled, giving 198 data points for analysis. Mean resin volumes shown by treatment group and time period are represented in Figure 14. Pre-treatment resin flow was not significantly different between control trees ($\bar{x} = 6.3$ mL) and char treatment trees ($\bar{x} = 4.5$ mL), as anticipated with randomly assigned treatments among trees of relatively homogenous vigor. In addition, pre-treatment resin flow was not significantly affected by *Height* or the *Trt:Height* interaction.

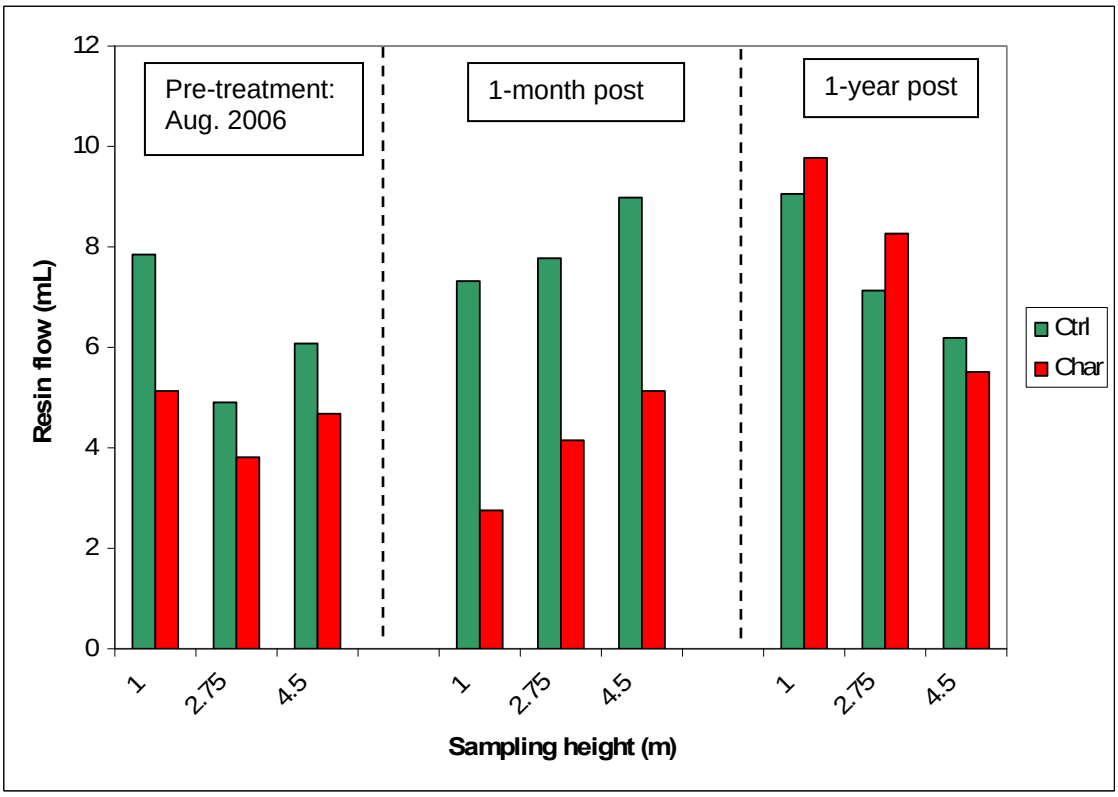

Figure 14. Summary of mean resin volume at the three time periods in the two treatment groups.

As previously described, post-treatment resin flow was modeled using differences in resin volume from pre-treatment levels at the 1-month and 1-year time periods. In the initial full factorial model, the *Trt:Height:Time* 3-way interaction, the *Trt:Height* interaction and *Height* main effect were all non-significant; of the terms containing the *Height* variable, only the *Height:Time* term was significant. The pattern is evident from Figure 14: the relationship between post-treatment resin volume and height is clearly different over the two post-treatment time periods – at 1 month post-treatment, resin volume increased with height, and at 1 year post-treatment, resin volume decreased with height. Since this resin-height relationship is equally evident in control trees and treatment trees (as indicated by the non-significant 3-way interaction term), it was likely caused by factors external to the factors of interest here. Based on non-significance of these terms, the *Height* variable was dropped from subsequent model-fitting.

The simplified post-treatment resin model showed the *Trt* main effect factor was not significant at the α =0.05 level, indicating similar overall changes in resin flow volume averaged over the two post-treatment times. However, both the *Time* main effect and the *Trt:Time* interaction were significant, reflecting both overall differences in resin volume change at the two time periods and the opposing effects of the *Time* factor on the two different treatments. Because the two remaining predictor variables are categorical, parameter estimates can be easily added together to give group means. Thus, control trees had slightly increased resin flow at time 1 (after 1 month: $\bar{x} = +1.7$ mL, $p = 0.019$) and time 2 (following year: $\bar{x} = +1.742 - 0.576 = +1.2$ mL, not significantly different from +1.7 mL); meanwhile, charred trees had a slight decrease after 1 month ($\bar{x} = 1.742 - 2.273 = -0.5$ mL; $p = 0.015$, significantly different from control group at time 1) and a stronger increase the following year ($\bar{x} = 1.742 - 2.273 + 4.409 = +3.3$ mL; $p < 0.001$, significantly different from charred trees at time 1), compared to the increase in

the control group. Figure 15 shows the summary of resin flow at the three measurement times, organized by group (excluding height differences).

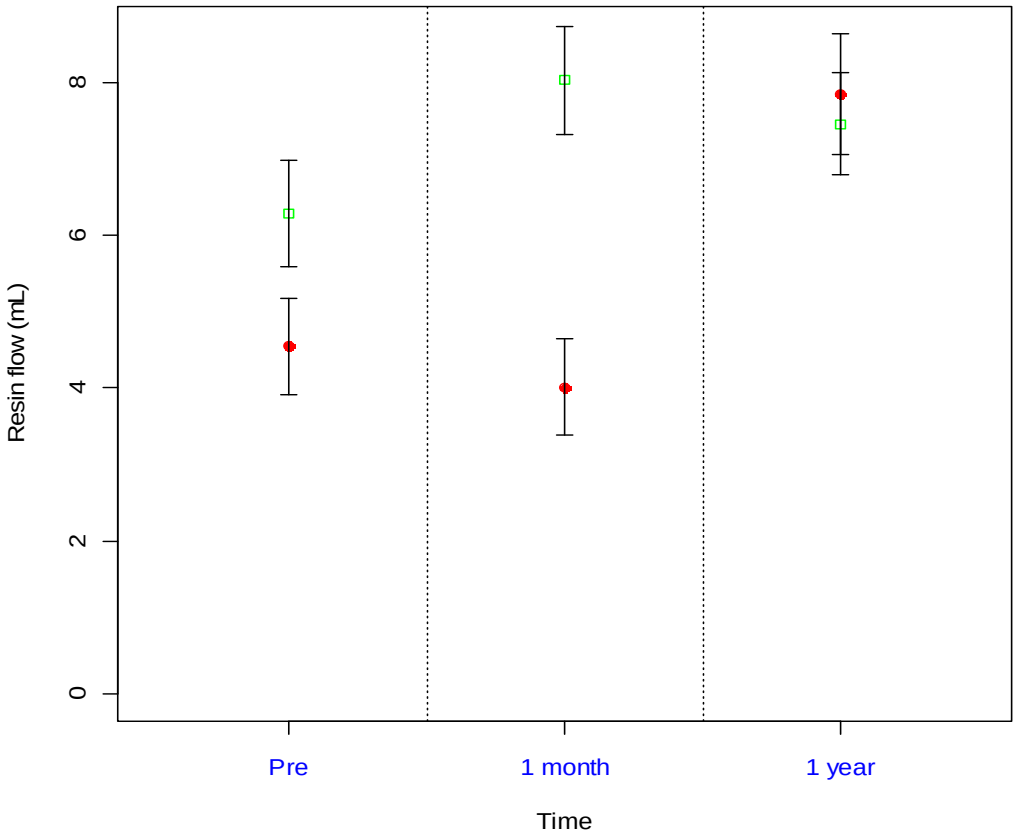

Figure 15. 24-hour resin volumes on all trees (ignoring height effects), by time and treatment group. Symbols represent treatment means (green: control; red: charred) and whiskers are standard errors.

Experiment 5. Fire effects on resin chemistry

Oleoresin plays an important role in conifer defenses, with evidence suggesting that both resin quantity and composition affect tree defenses. Experiment 5 focused on potential changes to resin composition following prescribed fire, focusing on the monoterpenes – the resin fraction known to be volatile, toxic (to beetles), and highly important in beetle semiochemistry. Techniques for separating and identifying monoterpenes are well-developed and dependable using modern laboratory techniques.

The present analysis was relatively simple and exploratory in nature. Although resin chemistry is a complex topic that could be explored in depth – examining monoterpene enantiomers separately, for instance (Pureswaran et al. 2004b) – this experiment consisted of a simple collection of resin from burned and unburned trees at two separate study sites. Monoterpene analysis was done using gas-liquid chromatography. Special emphasis was placed on limonene, 3-carene, and myrcene due to the importance of these three monoterpenes in western pine beetle chemical ecology (Smith 1972b, 2000).

Monoterpene composition changes were assessed based on resin collected from 46 trees at two locations, each with very different site characteristics: The first site was Sun Pass, OR, approximately 1 km from the Experiment 3 location, and subjected to a spring prescribed burn in 2006; the second site was in the Preston Creek drainage, in the Entiat River watershed near Ardenvoir, WA, and subjected to a fall prescribed fire in 2006. The Sun Pass area was previously described in Experiment 3, although the stand selected for this experiment was composed of larger, mature size ponderosa pines located in the thin land section managed by Winema National Forest adjacent to Crater Lake NP and Sun Pass SF (see Figure 3, WINF site). The Preston Creek site was located in the eastern Washington Cascades in an area with a history of at

least two large wildfires in recent memory – the 1970 Entiat fire and the 1994 Tyee fire, both being landscape-level high intensity events; the stand was also treated with a lower-intensity prescribed fire in October 2005. The specific stand selected for this experiment was uneven-aged, including younger pine individuals (ponderosa and lodgepole) that likely germinated following the 1970 event as well as older veterans of larger size- and age-classes (many with fire scars); other tree species observed in the drainage included Douglas-fir and trembling aspen.

Resin was collected from burned and unburned trees in both sites in September 2007. At the Sun Pass site, usable samples were obtained from 25 trees (13 burned, 12 unburned). Two unburned trees failed to produce any resin at all during the sampling period. At the Entiat site, 24 trees were originally selected for sampling; 3 unburned individuals yielded no resin, resulting in viable samples from 21 trees (12 burned, 9 unburned).

Methodology for resin collection was similar to Experiment 3 with minor differences: in each tree, a 5.56 mm (7/32 inch) hole was drilled close to the ground (10 - 20 cm height) at an upward angle into the sapwood. A brass tubing funnel directed emergent resin into a 4 mL glass vial (Entiat) or centrifuge tube (Sun Pass). Resin was exposed to open air for 1-5 hours, as required to obtain a sample of at least 1 g. Following collection, vials were capped and kept on ice for 1-2 days before being transferred to a laboratory freezer for storage prior to analysis. Unfortunately, the Sun Pass samples had to be transferred from centrifuge tubes to glass vials prior to analysis. This involved leaving them to warm to room temperature before transferring the resin (pouring and scooping from the tubes to vials. At this time it was noted that considerable resin crystallization had occurred.

Monoterpene analysis was conducted at the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, Flagstaff, AZ). Resin samples were dissolved in hexane, vortexed,

and then left to stand for 24 hours to ensure resin dissolution. A known volume of sample (30-75 μL) was measured using a gastight SGE syringe and transferred into a 5 ml volumetric flask containing 50 μL of standard fenchone. The sample and internal standard were diluted with n-hexane to the 5 ml mark. Samples were transferred to analysis vials using a glass Pasteur pipette.

Analysis was performed on an HP5890 gas chromatograph (HP-Agilent Technologies, Santa Clara, CA) equipped with a DB-1 capillary column (J&W Scientific, Folsom, CA) and an HP Automatic Liquid Sampler. On-column inlet temperature was 275°C and the helium flow pressure was set to 10 PSI. The following temperature program was used: 40°C for 10 minutes, increase to 165°C at a rate of 2°C/minute, hold for 0.5 minutes, increase to 300°C at a rate of 20°C/minute, hold 2 minutes. Eluting was measured with a flame ionization detector (FID) set at a temperature of 300°C. Peaks were identified using retention time on the chromatogram and quantified with a calibration curve developed from standard laboratory chemicals. PeakSimple chromatography software (SRI Instruments, Torrance, CA) was used to measure and record data.

The first analysis represented the difference in total proportion of monoterpenes (as a fraction of total resin mass) between burn and control treatment groups. Difference between the means was analyzed using a two way analysis of variance (factors *Site* and *Treatment*). Individual monoterpene data were analyzed using the same 2-way ANOVA model on the differences in monoterpene proportion (per mille (‰) of total monoterpene mass) between sites and treatment groups.

Overall, the mean monoterpene fraction in resin samples was 246.8 mg/g resin, with a standard deviation of 66.8 mg/g; thus, approximately one quarter of the resin weight from all samples consisted of the monoterpene component. Despite the short exposure time during sampling (~2-5 hours total), resin crystallization was observed on the walls of several sampling

vials during collection and storage, suggesting that resin in these samples was reacting with ambient air, with monoterpenes volatilizing. Among the control trees at both sites, monoterpene proportions were similar (p=0.582). However, the two-way ANOVA (factors *Site* and *Treatment*) had a significant *Site: Treatment* interaction effect: at Entiat, burned trees (307.21 mg/g resin) had higher monoterpene proportions than control trees (235.33 mg/g resin), while at Sun Pass burned trees (160.4 mg/g resin) had lower monoterpene proportions than controls (223.22 mg/g resin).

The GLC analysis tested for and detected 7 monoterpenes in various proportions in the samples. In terms of ranking, the most abundant monoterpene was δ-3-carene, followed by myrcene, followed by β-pinene (absent from a few samples), followed by a tie between α-pinene, limonene and terpinolene (Table 2); fewer than half of the samples (mostly those from the Sun Pass site) also had trace levels of longifolene. Relative ranking of monoterpene compounds is affected by some of them being absent (or below detectable limits) from some samples.

Proportions of all monoterpenes varied considerably between individuals, with few patterns standing out. This experiment did not control for tree size, age, or pre-treatment resin properties – all of which could arguably confound real treatment effects. In any case, none of the tested differences were significant at the α=0.05 level. Limonene proportions were not significantly different between burned and unburned trees at Entiat (p = 0.363) or at Sun Pass (p = 0.151). Mean limonene proportions actually moved in opposite directions at the two sites, with slightly lower (N.S.) proportions in burned trees at the Entiat site, and slightly higher (N.S.) proportions in burned trees at Sun Pass. Likewise with myrcene – burned trees at the Entiat site had slightly lower (N.S.) myrcene levels than controls, while at Sun Pass burned trees had slightly higher (N.S.) myrcene levels than controls (Entiat: p=0.137; Sun Pass: p=0.359).

Differences in 3-carene proportions, the most abundant monoterpene, were similarly non-significant (Entiat: $p = 0.467$; Sun Pass: $p = 0.788$); slight decreases (N.S.) in mean 3-carene proportion in burned trees at both sites were lost amidst the high within-group variability (Figure 16).

Table 2. Mean proportions and ranking of monoterpenes at both sites (mg monoterpene/ g resin). Rank refers to the average rank within each sample of each monoterpene.

		α-pinene		β-pinene		Myrcene		δ-3-carene		Limonene		Terpinolene	
		‰	Rank	‰	Rank	‰	Rank	‰	Rank	‰	Rank	‰	Rank
Entiat	**Control**	9.36	4.3	11.49	4.6	42.94	2.2	149.94	1.0	10.84	4.7	10.02	4.1
	Burn	13.06	4.6	33.49	3.7	47.98	2.4	190.09	1.0	7.73	5.3	13.83	4.3
Sun Pass	**Control**	11.47	4.3	33.61	3.6	36.80	2.6	124.15	1.0	7.95	5.2	7.85	4.6
	Burn	13.70	4.6	23.03	3.9	40.75	2.3	120.43	1.0	14.19	4.2	7.91	5.0

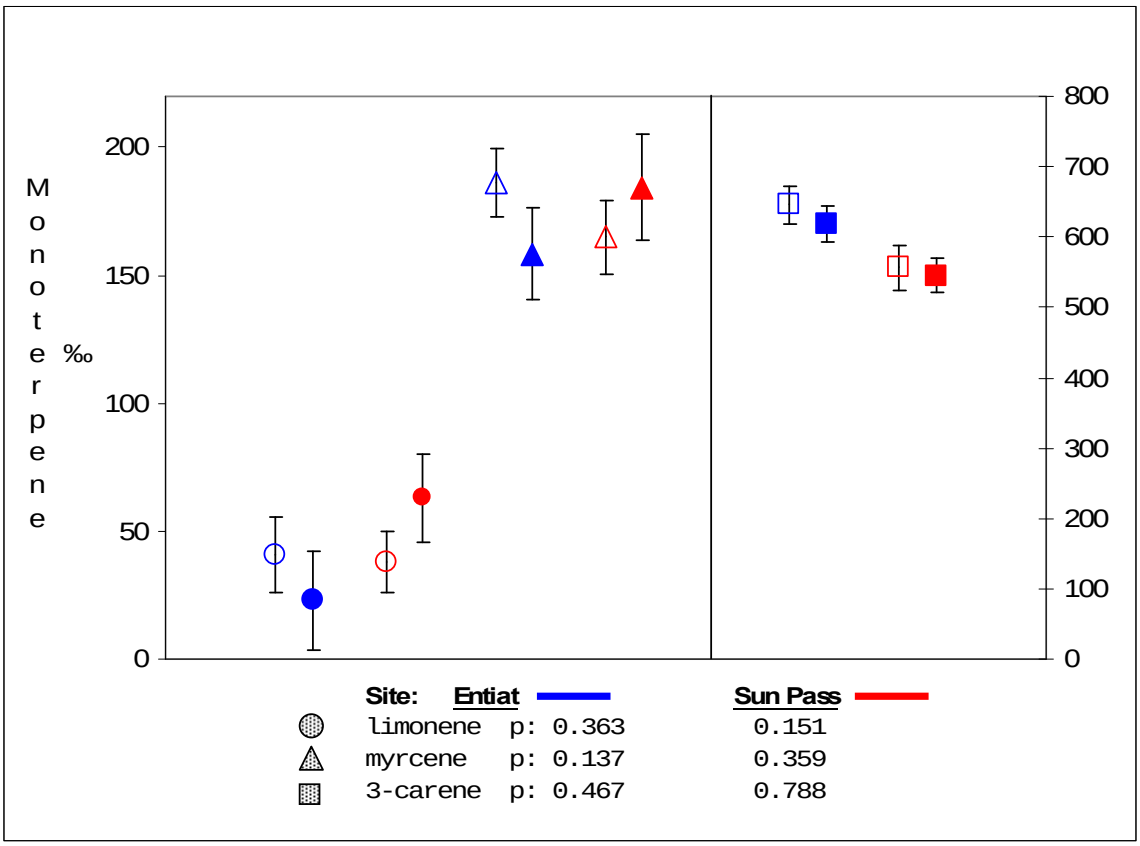

Figure 16. Normalized mean proportions (mg monoterpene/ g total monoterpenes) and standard errors (whiskers) of limonene, 3-carene, and myrcene by site and treatment. Proportion-values refer to t-test differences between treatments – control (open symbols) or burned (closed symbols).

Experiment 6: Methods testing between resin sampling techniques

This experiment was designed to test and quantify some of the variability in sampling methodology, as well as test certain assumptions regarding resin sampling in general. Since attacking bark beetles do not penetrate deeper than the outer face of the xylem (Miller and Keen 1960), there is some question as to the utility or validity of measuring oleoresin properties deeper in the sapwood (Lorio 1994). As briefly mentioned in Experiment 3, OEF methods that involved removing larger sections of bark have the disadvantage of causing much larger scars, potentially causing cumulative and severe injuries to sample trees. However, using an arch punch tool to cut through the bark and phloem has the advantage of avoiding injury to the xylem, since it is a relatively easy task for the operator to feel when the tool has penetrated the bark and phloem. In addition, the arch punch method only measures resin exuded from the outer face of the xylem (or phloem), which is potentially more representative of defenses encountered by a bark beetle than deeper in the sapwood. Thus, it has been suggested that this technique might provide a more realistic index of beetle susceptibility than drilling into the sapwood xylem (Lorio 1993, 1994). The counter argument is that vertical and radial resin canals are interconnected in continuous networks, and therefore, resin mobilized to wound sites can originate from deeper in the sapwood. Furthermore, resin pressure should be fairly constant within a tree (Vité 1961) as water can travel between sapwood xylem cells in all directions (Tyree and Zimmerman 2002). However, resin pressure and resin flow or volume are not equivalent, and as mentioned in previous sections, several authors have observed wide variations in intra-tree resin flow, particularly in the case of trees experimentally treated on only one side. This experiment was designed to test the assumption of equivalency between resin flow exuded from the xylem face

and from deeper in the sapwood; this was evaluated by measuring OEF using two methods simultaneously in individual trees.

Sampling for this experiment was conducted in two separate sampling periods and locations. The first iteration took place 24-25 September 2005 on 14 mature ponderosa pines widely scattered near Leavenworth, WA, along the Icicle Creek and Wenatchee River valleys. The arch-punch sampling method was previously described in Experiment 4. This first iteration and data set involved some protocol differences from those described previously, however: instead of using the Log Wizard tool to smooth the bark at the sampling location, bark thickness was shaved by hand using chisels, similar to the methods described by Lorio (1993). In addition, a 2.54 centimeter (1-inch) diameter arch-punch tool was used rather than the smaller 1.27 cm in previously described experiments. Finally, rather than using the enclosed resin samplers, resin was funneled from each circular scar to the centrifuge tube using small sections of sliced plastic tubing material that were pinned to the bark below the scar (Figure 9). The protocol for the drilled OEF method being contrasted was identical to that described in Experiment 3. Two arch-punch and two drilled resin samples were taken per tree, and resin volumes were verified the following morning (14-15 hours later).

The second iteration of this experiment took place September 15, 2007 in the Entiat River Valley, ~ 1 km from the location where monoterpene samples were collected (see Experiment 5). This time the Experiment 4 protocols were closely followed for the arch punch technique: bark shaving was accomplished using the Log Wizard tool, a 1.27 cm (0.5 inch) diameter arch punch was used, and plastic resin sampler attachments (Karsky et al. 2004) were screwed in place over the sampling scar (Figure 17). In addition, the trees being sampled were all pole-sized ponderosa pines that had been burned the year before in a fall prescribed fire (see Experiment 5). Again, the

drilled OEF protocol being contrasted was identical to the methodology described in Experiment 3. However, the sampling time period was very short – only 2.5-3.5 hours elapsed between the equipment installation times and the resin volume measurement times. To avoid any time bias between the two methods, drilling and phloem removal (with the arch-punch tool) were performed sequentially from tree to tree (two drilled samplers and two arch punch samplers installed on one tree before moving to the next tree).

In addition, this experiment section also describes the findings from a brief microstudy of ponderosa pine resin canals. Three small (< 20 cm dbh) ponderosa pines were cut from the Sun Pass site, and discs from each were sanded smooth with 800 grit sandpaper. Blocks from two of the trees' discs were cut so as to expose radial and transverse sections of the xylem, and then sanded as before. The sanded discs and blocks were examined for approximately 1 hour each at up to 200x magnification, with a particular focus on searching for any evidence of transverse resin canals.

Due to the differences in experimental protocols (primarily the 1.27 cm vs. 2.54 cm arch punch diameters used at the two sites), the two experiment iterations were first analyzed separately. Linear mixed-effects models were used in both cases in a split-plot (nested) design (Pinheiro and Bates 2000). Resin *Volume* measured at each individual sampler was the response variable since the objective was to test the variation between methods rather than between trees. Because 4 measurements were taken from each tree, *Method* was the main fixed factor, with measurements grouped by the random factor *Tree.* As before, raw resin volumes were square-root transformed to stabilize variances. Differences were evaluated at the α=0.05 level of significance.

Resin flow measurement using both the drilling and arch punch methods can also be thought of exposing a certain surface area of sapwood xylem to the air. Using simple geometric equations, the two resin flow methods were compared in terms of mL of resin collected per cm^2 of xylem exposed. Thus, the 1.27 cm arch punch tool exposed a circle of $\pi \cdot r^2$ (r is radius of the circle) or $\pi \times \left(\frac{1.27cm}{2}\right)^2$ = 1.267 cm^2. The value for the 2.54 cm arch punch is 5.07 cm^2. Modeled as a simple one-sided cylinder with height of 3.81 cm (based on an estimated hole depth of 1.5 inches, as described in Experiment 3 methods) and diameter of 0.556 cm, the drilled hole method exposes $\pi \cdot r^2 + c \cdot h$ (c is circumference, h is cylinder height), or $\pi \cdot (0.556/2)^2 + \pi \cdot 0.556 \cdot 3.81$ = 6.89 cm^2 of xylem area. These values do not account for the slight curvature on the face of tree boles, nor for the angled outer cylinder end for the drilled method; considering the limited precision of these techniques, these approximations are probably adequate. Based on this geometric model, resin yields using 3 different techniques (drilling, 1.27 cm arch punch, 2.54 cm arch punch) were compared using a simple bivariate correlation; to reduce within-tree error and simplify the analysis, resin quantities were evaluated as totals per tree using each method.

Between-tree variability differed between the two iterations of this experiment, with the Leavenworth site trees being much more variable than the Entiat trees. The main difference between sites is that the sampling with the smaller arch punch tool (Entiat) yielded significantly lower resin volume than the drilling method (p = 0.0063). At the Leavenworth site, the difference between the resin yield from the larger arch punch sampler and the drilled method was not significantly different (p = 0.800). These findings are consistent with the notion of a constant resin yield produced per unit of exposed xylem.

The exposed xylem model area produced a strong correlation between the two resin collection methods (slope = 0.922, $p < 0.001$, $r = 0.855$; Figure 17). Although some unexplained error remains, particularly around the Entiat sample values, this model supports the concept of resin flow measurements being largely determined by exposed xylem surface area.

The microstudy involved a very brief look at the xylem of three small ponderosa pines. Vertical and radial canals were readily observed and abundant, but there was no evidence of transverse canals. Although this was by no means an exhaustive or systematic analysis, it would appear that any transverse resin movement is much rarer, if even possible, compared with vertical or radial resin movement. This is in agreement with the Fahn's (1979) generalized observations that there are no transverse resin network in pines, and that resin canals exist only in vertical and radial 2-dimensional planes. Variable resin properties measured from different sides of individual trees (as reported in Perrakis 2004) are thus readily explained.

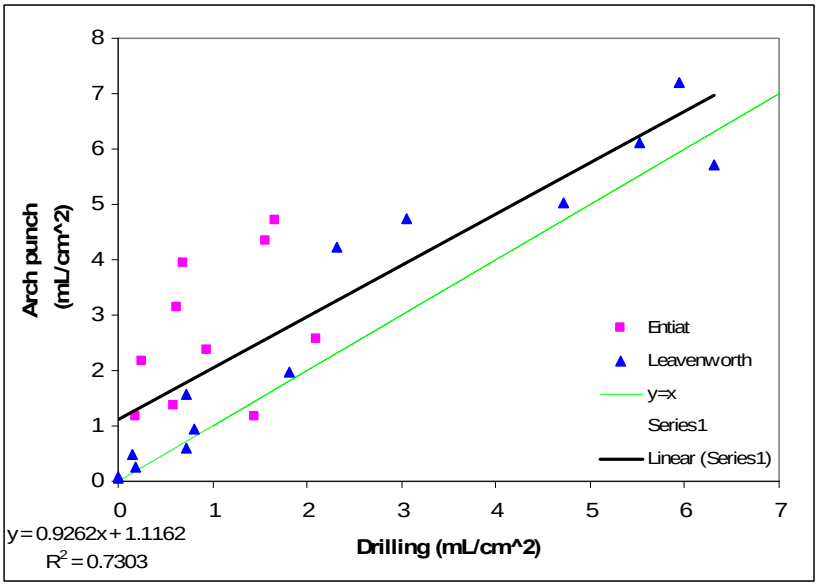

Figure 18. Xylem surface area model showing correlation between resin sampling methods. The Entiat sample (pink squares) used a 1.27 cm diameter arch punch on burned trees, and the Leavenworth sample (blue triangles) used a 2.54 cm arch punch on unburned trees. Each scatter point represents the resin volume for one tree. The green line is the line of perfect correlation ($y = x$) between methods, and the black line is a fitted linear regression using data from all trees.

Discussion and Management Implications

Monitoring Mortality of Old Pines and Resin Flow at Crater Lake

Monitoring tree populations over time can help assess the long-term effects and recovery following management treatments. Experiment 1 demonstrated that post-treatment mortality following prescribed fire in these forests can continue for several years. By the end of the monitoring period (4 years after burning), ponderosa pine mortality had declined in spring burn units to match the level associated with control units (by 2006, 2 trees died in each treatment type, 0.3% of pre-treatment populations). Mortality in fall units was declining steeply as well, although still elevated compared to controls (13 trees died in 2006, 2.4% of pre-treatment population).

Continuing the pattern found in previous surveys at this site (Perrakis 2004), mortality was strongly associated with Keen's (1943) crown class. Although this type of risk-rating system is very simple (involving only a visual estimate of crown vigor), it was also significantly (inversely) correlated with the number of rings in the outer centimeter in these trees. Thus, trees in A and B classes had higher radial growth in recent years than those in C or D classes, and the former were more likely to have survived for 4 years after burning, regardless of burn season or control treatment. It is to be expected that higher vigor should correspond with higher survivorship, as trees with well-developed root and crown systems tend to have high carbohydrate reserves and are more resilient to environmental stresses and pathogens attacks (Waring 1987, Kozlowski and Pallardy 1997a). The present findings corroborate this, with high crown vigor class being positively correlated with wider growth rings, higher resin flow, and lower mortality rates. Several previous studies in ponderosa (Larsson et al. 1983) and other pines

(Raffa and Berryman 1982, Stuart 1984) have also found higher radial growth indices to be positively related to tree survival following bark beetle attacks.

The mortality levels observed in this study (2.3 %, 6.1 %, and 16.4 % in control, spring burn, and fall burn treatments, respectively, after four years) are similar to or slightly lower than levels reported in other studies on ponderosa pine survival after fire. Thies et al. (2005) studied mortality in uneven-aged ponderosa pine stands (7.5 to >75 cm DBH) in eastern Oregon. They noted between 0-0.6 % annual mortality in control plots, and mortality in burn plots that peaked one year after burning (spring burns: 4.3 %, fall burns: 11.8 %) and leveled off (matching control levels) after four years of post-burn monitoring – a nearly identical pattern to that observed in the present study. Their fall burn treatments caused higher mortality overall (29 % of trees dead after four years), but the uneven-aged population structure suggests that many of the killed trees would be much smaller than those in the present study (Thies et al. 2005), and therefore susceptible to severe crown scorch (Harrington 1993).

As reported previously (Perrakis 2004), earlier studies at Crater Lake observed higher mortality in spring burns than in fall burns (Swezy and Agee 1991, Agee 2003). However, a review of the literature on ponderosa pine mortality reveals a mix of findings on seasonal fire effects. Although phenology may suggest that early season burn effects are most likely to be lethal due to reduced carbohydrate stores (Kozlowski 1992), prescribed burns later in the season tend to have higher fire intensities and proportional coverage (Ganz et al. 2003, Perrakis 2004, Knapp and Keeley 2006, Schwilk et al. 2006). A recent study comparing spring and fall burning in a Sierra Nevada mixed-conifer forest found that ponderosa and sugar pine mortality were significantly related to fire intensity, while mortality was not significantly different overall between the two burning seasons (Schwilk et al. 2006). In northern Arizona, McHugh and Kolb

(2003) compared spring and early summer wildfires to a fall prescribed fire. They observed that the most significant predictors of mortality in logistic regression models were crown damage (scorch and consumption) and bole char – factors closely related to fire intensity. In the present study, with spring burns having significantly lower coverage and visibly lower intensities than fall burns (Perrakis 2004), a similar pattern emerges. Although season of burn and tree phenology should not be discounted, the fire intensity of a particular fire is probably a more important variable overall in predicting mortality.

For management purposes, it is promising that mortality has virtually dropped off to control levels after four years, even in the intense fall burn treatments. Thirteen years after a series of very similar burns at another Crater Lake site, Agee (2003) found no significant difference between large ponderosa pine mortality in burned units and unburned controls. Ideally, post-burn mortality should be below control unit mortality levels after a few years. Since these sites have been identified as suffering from low forest health due to large increases in tree density and competition in the fire-exclusion era (McNeil and Zobel 1980, Agee 2003, Perrakis, unpublished vegetation data), restoration treatments are intended to promote increased survivorship among remaining old pines (Kolb et al. 2007). Reaching a point after a few years at which mortality levels appear equal between burn treatments and controls is a good start, but is not indicative of long-term success according to the criteria of forest health restoration. Due to the generally low levels of mortality among untreated stands, even among stressed trees, identifying enhanced post-treatment survivorship will require longer-term monitoring efforts.

The Crater Lake burning and resin monitoring experiment presents one of the longest annually-repeated post-fire resin monitoring efforts in the literature. The pattern of increased post-fire resin flow identified in numerous other studies (Santoro et al. 2001, Perrakis 2004,

Knebel and Wentworth 2007) is hereby confirmed to continue for up to four years after fire. The results suggest that four years after burning, constitutive and induced resin defenses may still be higher than those in unburned controls. The relative increase is clearly declining, however, and may be expected to reach unburned levels within another year or two (years five or six after burning).

Absolute resin flow was also greater in trees of high crown vigor class. As crown vigor class was inversely correlated with rings per cm (Experiment 1), this suggests a positive correlation between constitutive resin flow and sapwood xylem production (although this was not tested in the same trees; cored trees and resin-tapped trees were different samples). While this does not support the GDB predictions (see below), it is in agreement with the findings of McDowell et al. (2007), who found positive correlations between basal area increment and resin flow in a survey of several different studies in southwestern ponderosa pine stands. Although the BAI factor was not significant in any of the analyses in Experiment 1, that may have been due to small sample sizes.

Fuel Buildup

The burn treatments successfully reduced both fine and total fuels, and differences were still statistically significant by 2005. However, the benefits in terms of reduced fuel loading were relatively limited, particularly in spring burn units, and likely short-lived as fire-killed biomass progressively becomes part of the surface fuels layer in the immediate post-fire years. As previous studies have suggested, a first post-exclusion restoration burn will often kill but not consume vegetation, reducing the fuel-reduction effectiveness of the treatment (Thomas and Agee 1986, Agee and Huff 1987); in the case of a dense forest with many trees killed, dead

surface fuel loads can be expected to surpass pre-burn levels within a few years after fire, if the treatment is not renewed.

Standing fire-killed snags will eventually fall and contribute to coarse surface fuel loading, with larger-diameter snags generally lasting longer than smaller-diameter ones (as reviewed by Everett et al. 1999). Loading of finer materials tends to increase after fire as well, and may peak and then drop if decomposition surpasses inputs of new material, in the absence of another fire event. In the Colorado front range, Hall et al. (2006) found that total woody debris levels in ponderosa pine forests peaked approximately 10 year after individual (primarily high severity) fire events before declining for several decades afterwards. For litter and duff layers, the response after a single fire appears asymptotic, with no clear peak discernible even after many years. Mackenzie et al. (2004) found that approximately 2/3 of the forest floor had returned 10-15 years after fires in ponderosa pine/Douglas-fir forests in western Montana, with no evidence of peaking even 130 years after fire (severity or consumption levels of fires were not specified, unfortunately). Fernandes and Botelho (2003), citing several articles from locations around the world, suggest that litter fuels tend to recover even quicker – approximately 2-5 years after typical prescribed fire treatments in a number of different ecosystems.

A recent publication of long-term fuels monitoring work in the Sierra Nevada range is probably the most comparable to this study (Keifer et al. 2006). Pre-burn fuel levels (and tree species composition) were quite similar to conditions at Crater Lake, with 163-191 Mg/ha of total fuels measured before burning. Total fuel loading in ponderosa pine forests, initially reduced by 99 % by prescribed burning, had recovered to 85 % of pre-burn fuel levels after 10 years. A separate series of plots in different ponderosa pine vegetation associations was monitored after burning, and had surpassed pre-burn levels – by 57 %, 80 %, and 50 % – 31

years after prescribed fire. In white fir/mixed-conifer forests, fuel loading had returned to 83 % of pre-burn levels 10 years after burning, despite being reduced by 85 percent immediately after burning. Post-fire fuel composition tended to be different than pre-treatment conditions, with higher levels of woody fuels and lower levels of duff post-burn (Keifer et al. 2006).

In terms of operational effectiveness, anecdotal evidence has suggested for many years that prescribed burning, with or without mechanical thinning, could be effective in mitigating subsequent wildfire severity and crown fire likelihood in ponderosa pine forests (reviewed by Finney et al. 2005). Several rigorous studies have now confirmed that suggestion in several western forest types (Pollet and Omi 2002, Finney et al. 2005, Raymond and Peterson 2005). Of particular relevance is the study by Finney et al. (2005), which found that fuel treatments, including prescribed burns, conducted up to 9 years prior effectively reduced the severity of the 2002 Rodeo-Chediski fire complex – the largest wildfire in Arizona in modern history. However, Southwestern ponderosa pine forests tend to have lower overall surface fuel levels than those in this study (e.g. Fule et al. 2002); annual productivity of these forests are also quite different. It should not be assumed that the findings of Finney et al. (2005) apply equally well to the ponderosa pine forests at Crater Lake.

In sum, by 2005 the 2002 prescribed fires at Crater Lake still appear somewhat successful at lowering fuels from fire exclusion levels. However, it is clear that biomass inputs from killed and scorched trees are undermining the gains achieved thus far. Managers can expect total fuel loads to return quickly to pre-burn levels, probably within about 10 years or less. In the long term, dead fuels in burned areas will likely surpass levels in unburned areas if additional burns are not conducted to further restore and maintain low severity fire.

The findings in this component of this study have focused on dead surface fuels, which are only one part of the fuel complex that determines wildfire behavior. Canopy fuels and structure are equally or more influential in determining the intensity of crown fires (van Wagner 1977, Scott and Reinhardt 2001), although surface and canopy fuel production are related, since post-fire increases in dead surface fuels are withdrawn from existing canopy fuel loads. Additional modeling efforts on changes to forest structure and implications for fire behavior would be needed to properly assess the dynamic changes due to restoration or fire-safe treatments over time. While fuel management objectives may only require additional prescribed burns, managers will also have to balance fuel reduction with the goal of maintaining old ponderosa pines at these sites, as discussed in the main body of this study. Fire regime restoration at Crater Lake will require continued commitment and long-term investment of time and resources if it is to succeed.

Fire Surrogate Treatments and Resin Flow

Prescribed fire effects on resin flow were investigated via controlled injury treatments to tree structures. Root trenching and lower crown pruning comprised the injury surrogate treatments in Experiment 3, and bole heating was the surrogate treatment in Experiment 4. Various tree growth and size covariates were also tested for potential effects on resin flow. The mixed-effects models had many terms and interactions, and had the potential to be complex to untangle and interpret. In retrospect, Experiments 3 and 4 had much in common with another recently published study, Lombardero et al. (2006), which studied the effects of bole heating and mechanical wounding on resin flow in red pine.

In Experiment 3, most model terms were non-significant, resulting in fairly straightforward model interpretation and findings. Based on 30 sampling sessions over 3 years after treatment, there was no evidence that any of these mechanical injury mechanisms had significant effects on resin flow, as evaluated against untreated controls and a prescribed burn. Of the four treatments contrasted with controls in the various analyses, only the burning treatment showed significant deviation from default resin flow patterns. None of the physiological covariates related to tree or crown size, growth rate, or carbon-13 discrimination were significant predictors of resin flow in any of the analyses, before or after treatment. Seasonal patterns of resin flow in this study were similar to previous research in pine species (Harper and Wyman 1936, Lombardero et al. 2000, Wallin et al. 2004), with the highest resin volumes obtained in mid-summer (July-August), and lower yields earlier or later in the season.

With respect to the burned trees, there was evidence of increased post-fire resin response one or more years after burning, as has been previously documented in ponderosa pines subjected to prescribed burning (Kolb et al. 1998, Perrakis 2004). In this study, there was also evidence of a short-term reduction in resin flow measured a few weeks after burning – a response that was hypothesized, but not confirmed, in the previous work at Crater Lake (Perrakis 2004), and that was also observed in a similar experiment on red pine. In the latter study, Lombardero et al. (2006) found significantly lower resin flow (measured over 6 h) after 3 and 9 days on the treated sides of tree boles heated with a propane torch compared with untreated sides or on control trees.

The mechanism behind this reduction is unclear. Lombardero et al. (2006) suggested that short-term resin flow reduction may be a sort of 'shock response' to the treatment, similar to previous reports of lightning-struck trees readily succumbing to beetle attacks (Blanche et al.

1985). Physiologically, bole charring may have interrupted sap flow in bole tissues, ultimately leading to reduced resin pressure and exudation. Water potential has been found to be positively related to resin pressure (Vité 1961, Hodges and Lorio 1971), and fire damage to sapwood tissue might have increased water potential (lowering moisture stress) in the lower bole, reducing resin exudation pressure and volume in the days following burning. This mechanism assumes a positive correlation between resin flow and resin pressure at any one time, a correlation that was previously believed to be significant based on capillary flow modeling (Bourdeau and Schopmeyer 1958, Vité 1961) and found significant (although weak) in separate OEP and OEF measurements at Crater Lake (Perrakis and Agee 2006), but has been previously disputed (Lorio 1994).

Another mechanism for explaining the short-term resin flow reduction may simply be related to resinosis at injury sites. Bole charring can sever and expose sapwood resin canals (Ryan 2000), and resin loss from these wounds sites may have resulted in reduced resin volume and pressure within the canal network surrounding char wounds, and less constitutive resin remaining for sampling. Resinosis on the lower bole areas of burned trees was observed in this experiment (Experiment 3) as well as in prior burn experiments at Crater Lake.

Experiment 4 confirmed that bole wounding alone, with no accompanying forest floor disruption, root mortality, or crown scorch, caused a similar dynamic response in resin defenses. As in Experiment 3, charred trees in Experiment 4 had short-term reductions in resin flow one month following the treatment, and strong increases the following year. This experiment also highlights the importance of incorporating pre-treatment data into resin flow measurements, as the second year increases in resin flow were only significantly different from controls in terms of change from pre-burn values – not in terms of absolute resin flow volume.

It was previously hypothesized (Perrakis 2004) that a reduction in resin defenses (flow or pressure) following fire might provide a 'window of opportunity' for beetles to attack burned trees. Although Experiments 3 and 4 suggest that this reduction does occur, the timing of the resin decline does not match with observed patterns of beetle attacks. Increased post-fire beetle attacks have been found to peak 1-2 years after burning (see below) and continue for several years thereafter – far longer than the few weeks of reduced resin defenses suggested by this study. If this short-term reduction in resin flow affects the probability of beetle attack success in any way, it appears overshadowed by other factors in subsequent years.

The results of Experiments 3 and 4 suggest that post-fire resin flow increases are a response to bole injury, rather than to root or lower crown damage. This assumes that mechanical treatments effectively simulated fire effects to roots and crowns, an assumption that merits some discussion. Pruning treatments were simple to implement, and likely replicated lower crown scorch effectively. As in the case of a vigorous surface fire, the pruning treatment removed significant portions of the live leaf area in the lower crown, while leaving the upper crown unaffected. Treatment effectiveness was simple to assess, as the crown mid-point was identified prior to pruning, and branches were removed upwards until the point was reached. Growth-differentiation balance principles suggest that greater leaf area will provide a greater source of carbohydrates (assuming unchanging sapwood area and root mass), which is then available for growth or metabolic processes depending on other source-sink relationships (see below). However, the lower crown, which would be the most likely to be scorched by fire and was the target of pruning in the present study, is considered photosynthetically inefficient due to internal shading, compared to the upper and middle crown areas (Helms 1970, Kozlowski and Pallardy 1997a). Pruning of the lower crown may have even increased the trees' xylem water potential

(lowering moisture stress by decreasing transpiration sinks from foliage) without significantly reducing carbohydrate production. However, Ryan (2000) conducted a similar experiment (scorching lower crown foliage) on young (14-25 cm DBH) ponderosa pines, and noted no significant differences on transpiration or stomatal conductance following scorching treatments applied to 0%, 40% or 80% of crowns. He further suggested that his sample trees were likely resilient to foliage reductions due to carbohydrate stores in roots, and had apparently succeeded in regenerating foliage in the next few years. A similar resiliency may have existed in the pruned trees in the present study (root stores compensating for foliage reduction), with little measurable effect on either overall carbohydrate allocation or mid-summer water balance. Confirming this premise would require further testing. In the case of fire, young ponderosa pines are generally highly resistant to crown scorch (Dieterich 1979, Harrington 1993), although post-fire bark beetles attacks undermine that resistance in older stands, as previously discussed. In this study, data from Experiment 3 do not suggest any consistent effects of pruning on resin flow. There is no evidence that crown scorch effects include measurable changes to resin defenses that would affect bark beetle attack success.

Effects of root trenching were more difficult to assess. Prescribed fire effects in fire-excluded ponderosa pine stands can be severe, as thick duff and poor soil moisture retention promote dense root growth in the upper soil and forest floor (Swezy and Agee 1991). Although the soil parent material (pumice from the Mount Mazama eruptions) was identical at the Sun Pass site, duff layers appeared thinner at Sun Pass than at Crater Lake's 'panhandle' sites (based on unpublished Sun Pass soil pit data and Swezy 1988). This may be due to forest floor disturbance during recent management activities, such as entries into the stand for pre-commercial thinning (Ed Deblander, Sun Pass State Forest, personal communication 2003).

Alternatively, rates of organic material accumulation may have been lower in the second growth stands at Sun Pass, following logging of older trees in the mid-20th century, than in the old growth stands at Crater Lake. Thus, root growth at Sun Pass may have been less concentrated at the surface compared to the Crater Lake sites.

Aside from digging a soil pit in the stand (which confirmed that the upper mineral soil layers had abundant roots), pre-treatment root structure was not assessed. It was recognized that the effects of 'light' or 'heavy' trenching around the circumference of trenched trees would be highly variable. Such variability likely occurs in the case of actual fire effects on roots, but in this case (perhaps compounded by the small sample sizes) made the effects of treatments uncertain. The two levels of trenching (roughly 1/3 and 2/3 of treated trees' circumference) were chosen to attempt to induce two levels of moisture stress, potentially prompting imbalances in root:shoot ratios and perhaps reducing photosynthesis and carbohydrate availability. Although there was great uncertainty over the degree of root reduction required to stimulate changes to carbohydrate allocation, it seemed unwise to completely encircle trees with root trenches, lest they topple over due to lost stability. Neither treatment was a significant predictor of resin flow in any of the models.

The lack of significance of the carbon-13 discrimination factor suggests that moisture balance during the growing season was not a principal factor affecting resin flow. Previous studies have found strong relationships between $\delta^{13}C$ in xylem rings and moisture availability, due to the previously discussed stomatal responses and differential assimilation between ^{12}C and ^{13}C in photosynthetic tissue (see Experiment 3). Even if trenching treatments did not cause consistent effects in terms of induced moisture stress, $\delta^{13}C$ should still have emerged as a significant covariate in the resin flow models if it were a major resin flow predictor. Although

Experiment 3 suffered from small sample sizes and uncertain treatment effects, it does suggest that post-fire resin flow increases are not well simulated by mechanical damage to roots.

Resin chemistry and host-beetle interactions

Fire effects on ponderosa pine resin monoterpenes were studied at the Entiat and Sun Pass locations, sites at the northern and southern end, respectively, of the eastern Cascades. While these sites are about 500 kilometers distant from one another, the more important distinction may be between the trees' evolutionary history and adapted resin chemistry in each region. Following extensive study of ponderosa pine populations across North America, R. H. Smith grouped regions by monoterpene constituents, with 10 regions and sub-regional variants covering the species distribution across the entire continent (Smith 1977, 2000). The Entiat site was located in his Cascade Northern ecoregion, while the Crater Lake/Sun Pass site was in the Sierra Pacific region. Monoterpene compositions in the two regions are mostly similar, except that the average limonene content (percentage of total monoterpenes) of individuals in the Sierra Pacific region is about 10%, while for the Cascade Northern it is only 1%.

Much of the career of R.H. Smith was spent on the genetics and breeding of pest-resistant pine trees. His findings and observations suggested that limonene content was of major importance in determining the insect- and pathogen-resistance of individual ponderosa pines, and much effort was invested into studying, and ultimately breeding, trees with 'high-limonene' alleles (Smith 1972b, 2000). However, it is unclear whether the findings from various studies on individual monoterpenes are additive. Thus, although the studies mentioned above suggest that the most toxic monoterpene compositions would have high levels of limonene and δ-3-carene, and lower levels of myrcene and α-pinene, this premise is untested, and ignores the effects of

monoterpenes as pheromone precursors in many beetle species (Byers 1995, Seybold et al. 2006). Monoterpene combinations can also elicit different responses from beetles than exposure of lone monoterpenes in laboratory settings (Byers 1995), and other volatile compounds may also have unknown effects. Ethanol has been suggested as being potentially implicated in beetle host-selection after fire (Kelsey and Joseph 2003), although more studies are needed to confirm a mechanistic relationship between ethanol and beetle attraction.

In addition to limonene, other monoterpenes also have documented roles in western pine beetle host selection and ponderosa pine defenses. Myrcene, despite being a relatively minor oleoresin constituent (Smith 2000), was identified to be linked to ponderosa pine attractiveness by Bedard et al. (1969), who noted significantly higher attraction to the synthesized attractant pheromone *exo*-brevicomin in the presence of myrcene, compared with samples of the pheromone alone. Other experiments confirmed and refined this effect (Vité and Pitman 1969, Wood 1972), reporting that the most attractive western pine beetle pheromone consists of a mixture of beetle-synthesized *exo*-brevicomin and frontalin, along with myrcene from oleoresin. The monoterpene δ-3-carene is the most abundant monoterpene in ponderosa pine resin (by far), and is reported to be the second most toxic to western pine beetles, after limonene (Smith 2000). Alpha-pinene, considered the least toxic of the major monoterpenes (Smith 2000), was found to be a precursor to the western pine beetle anti-aggregant pheromones *trans*-verbenol and verbenone (Byers 1983), pheromones released by male beetles to reduce intra-specific competition on mass-attacked trees.

Monoterpenes are also significant in the chemical ecology of other beetles parasitizing ponderosa pine. Red turpentine beetles are highly attracted to several resin monoterpenes alone (a true primary attraction effect), including certain enantiomers of α-pinene, β-pinene, and δ-3-

carene (Hobson et al. 1993, Erbilgin and Raffa 2000). As reviewed in the Introduction, other scolytids that parasitize ponderosa pine, including mountain pine beetle and various *Ips* species, have also demonstrated responsiveness (such as antennal responses and preferential landing) to various ponderosa pine monoterpenes, often in combination with pheromone emissions. However, the particular monoterpenes that are detected vary considerably by beetle species and so no individual monoterpene or combination stands out as especially important for either resistance or susceptibility (Trapp and Croteau 2001).

In this study, the average proportion of monoterpenes across all samples as a fraction of resin mass was about 24.7%. This figure is consistent with Smith (2000), who reported a proportion of 25% monoterpene in ponderosa pine oleoresin across the continent. However, differences between monoterpene proportions were significant between sites and treatment groups, with a somewhat surprising interaction effect. At Sun Pass, burned trees had lower monoterpene proportions than controls, while the reverse was true at Entiat. This discrepancy is somewhat puzzling. The sole methodological difference between the two sites was that the Sun Pass samples were first collected in centrifuge tubes before transferring to glass vials, while the Entiat samples were collected in glass vials directly from the field. It is likely for the Sun Pass samples that some of the monoterpenes would have vaporized during transfer between containers (when the samples were exposed to open air, a process that took up to several hours). However, this does not explain why this effect would have been greater among burned samples than among controls. Resin monoterpenes are all hydrocarbons with the chemical formula $C_{10}H_{16}$, with very similar physical and chemical properties (Phillips and Croteau 1999).

It is tempting to suggest that the extra difficulties with the Sun Pass samples may have biased these data, rendering them invalid. This would leave only the Entiat samples as

appropriate for comparing treatment differences. A simple t-test between sample groups from the Entiat site (not shown previously) suggests a significant treatment effect ($p = 0.029$), with a greater monoterpene component in burned trees ($\bar{x} = 307.21 \pm 30.46$ mg/g) than in controls ($\bar{x} = 235.33 \pm 23.03$ mg/g). Both the implications and mechanism behind this effect, if it actually exists, are not clear. The wound-induced resin effect has been studied intensely in recent years on grand fir, where a very rapid wound response process has permitted intense biochemical study of induced resin synthase activity. Monoterpene synthases began almost immediately (detectable within 2 h) and were dominated by limonene, following wounding to saplings in laboratory conditions. Synthase activity of resin acids (diterpene) synthase began only 3 days later (Steele et al. 1998, Trapp and Croteau 2001). After 7 days, pinene synthase activity (indicative of α- and β-pinene) became most dominant. There may be little merit to comparing these two conifer species (grand fir and ponderosa pine), as they are the two extremes of evolved resin defenses – the pines with their elaborate constitutive resin canal networks, and grand fir, with an almost entirely induced defense system. Potential post-injury increases in ponderosa pine monoterpene proportions would add weight to the notion of induced or enhanced primary attraction in this species following fire, but has not adequately been demonstrated by Experiment 5. Further study should incorporate pre-burn resin composition data, which can be highly variable between trees even within a stand. Similar work to the elaborate studies on grand fir will likely be more problematic in ponderosa pine, due to the lack of rapidly inducible resin defenses and cyclase activity in this species (Lewinsohn et al. 1991).

The two fire sites examined in this study gave no indication that monoterpene composition (proportion of myrcene, 3-carene or limonene) was affected by fire injury. A few studies have previously noted various effects of stress on resin monoterpene composition,

although none have directly studied ponderosa pine resin composition following wounding or fire injury. Smith (2000) found that ponderosa pine saplings subjected to grafting, constrained root growth in containers, or heavy herbicide application responded with decreased levels of myrcene and limonene, and increases in α- and β-pinene. This combination of effects has not been identified to cause a net increase or decrease in either tree resistance or beetle attraction. Latta and Linhart (1997) found weak but statistically-significant positive associations between radial growth and myrcene, α-pinene, and terpinolene; however, negative bivariate correlations between individual monoterpenes made these findings inconclusive. Thoss and Byers (2006) found no significant differences in monoterpenes extracted from wood between narrow growth rings (produced in drought years) and wider-spaced growth rings. They also observed that trees lacking in one or more of the common monoterpenes (thus, having less common monoterpene compositions) seemed less likely to have succumbed to beetle attacks, but this observation was not statistically significant and is inconclusive without further testing (Thoss and Byers 2006).

Several studies have also examined effects of wounding on ponderosa pine foliage chemistry, such as due to herbivory or defoliation (Litvak and Monson 1998, Thoss and Byers 2006). However, these are not considered relevant due to known composition differences in resin from different structures in individual trees (Latta et al. 2000, Smith 2000).

Future research on the differences between constitutive and induced resin (Phillips and Croteau 1999) will be of greatest relevance to this question. As previously mentioned, there is some debate as to whether traumatic (induced) resin canals actually exist in ponderosa pine. In several other pine species, the canals that form around injury sites are indistinguishable from constitutive resin canals and only appear after delays of weeks or months (Bannan 1936, Fahn and Zamski 1970). In ponderosa pine, there is no short-term increase in monoterpene cyclase

activity (Lewinsohn et al. 1991) or measurable resin production (Wallin et al. 2003) following bole wounding. Exuded resin sampled several months or longer following stress or injury will typically include both constitutive and induced resin, precluding the simple study of injury response using common experimental methods.

The evolutionary interaction between western pine beetles and ponderosa pines has been described as an 'arms race' between trees and herbivorous insects (Seybold et al. 2000, Franceschi et al. 2005) – increasingly sophisticated host defenses have continually been matched by more complex and opportunistic host-selection techniques by beetles. The wide geographic range of ponderosa pine resulted in different selection pressures from beetles, and correspondingly different resin composition types. With respect to this study, there may have been different historic western pine beetle pressures between the southern (Crater Lake/Sun Pass, OR) and northern (Entiat, WA) study sites.

Seybold et al. (2000) attempted to synthesize beetle-host evolutionary adaptations for the entire family of scolytids. That review describes beetle attack strategies that have evolved redundancy to overcome conifer host defenses: beetles metabolize monoterpenes in the formation of pheromone attractants, but frequently have ways of synthesizing them *de novo* in biochemical terms – that is, without need of the monoterpene precursors. However, exploiting host compounds as precursors in these chemical reactions may conserve energy, helping promote their survival.

Focusing on primary attraction as a critical host-selection mechanism misses the point that in WPB, monoterpenes (esp. myrcene) are mostly used by beetles as co-attractants that greatly increase the potency of beetle pheromones (Seybold et al. 2006). Even if certain monoterpenes do not act directly as kairomones for western pine beetle, they may do so for other

beetle species. There is evidence for inter-specific pheromonal attraction in several species of scolytids (Pureswaran et al. 2004a), although none yet for *D. brevicomis*. Wallin et al. (2008) found other beetle species (incl. *D. valens*) attracted to western pine beetle pheromone, and speculate that the collective attacks by beetles in various positions on trees' boles overwhelmed tree defenses.

What of the growth-differentiation balance?

Since this study was initiated, several publications have weighed in on the question of the relationship between the growth-differentiation balance theory and resin production. Baier et al. (2002) and Rosner and Hannrup (2004) both studied factors affecting growth and resin flow in Norway spruce. The former noted certain findings suggesting agreement with GDB principles (specifically, negative correlation between radial growth indices and resin flow rate), while the latter concluded that resin flow traits were not conclusively related to environmental variation, but rather to genetic traits (the study was conducted using clonal saplings grown at different sites).

Results from a highly relevant study of ponderosa pine growth (aboveground and belowground production), photosynthesis, environmental variables, and resin flow were published recently, with specific reference to evaluating GDB predictions (Gaylord et al. 2007). The study reported high resin flow and low overall growth in 2002, a severe drought year; however, the following year, under more productive growing conditions (higher pre-dawn moisture potential [less moisture stress], slightly higher photosynthesis rates), growth of all structures was higher overall, as was resin flow. Overall conclusions from the study were that that GDB predictions were inconsistent in matching observed patterns of resin flow, as tradeoffs

between growth and resin flow (taken to signify carbon allocation to secondary metabolism) were evident in 2002 but not in 2003. The authors do point out that the study findings support GDB predictions somewhat if the first year conditions (2002; severe drought) are assumed to correspond to the left side of the photosynthesis-allocation curve (see Figure 1), and the following year conditions (2003; less drought stress) correspond to the peak of the curve. In that case, there is no need for tradeoffs according to the GDB model, as both growth and secondary metabolism can increase.

Methodological considerations

The last experiment involved a comparison of methods and assumptions behind resin flow measurements in this study. Based on the two trials in Experiment 6, the drilling method used in this study produced values closely resembling those of a 2.54 cm diameter arch punch. In addition, the geometric model based on the estimated xylem surface area exposed by each method gave a model with a reasonably strong correlation across both sites and arch punch sizes. Thus, there does not appear to be a qualitative difference between resin flow sampled from the sapwood to resin flow sampled at the cambium.

With respect to the xylem surface area model, some unexplained variability or error remains, particularly with the Entiat data. This variability may originate from differences in vertical canal density across the xylem, vertical canals being considered the main reservoirs of pine resin, their density positively correlated with resin flow (Blanche et al. 1992 and references therein). Resin canal density seems to vary during normal growing conditions based on environmental conditions (Blanche et al. 1992), but also wounding, pressure, and other stresses, including fire injury. Thus, the larger arch punch may be better suited to measuring an average

resin yield over a somewhat larger region of the bole that might be vary in resin production capability. Such variability might exist due to normal variations in environmental conditions during growth (Blanche et al. 1992) or, perhaps in this case, to heterogeneous stimulation due to fire injury.

As mentioned previously, the phloem removal/arch punch method has the advantage of clearly showing if tissue is alive at the collection site, and perhaps of limiting the damaged tissues to those affected by bark beetles. On the other hand, the drilling method creates a much smaller outer wound, and is simple to seal following sampling with wooden dowel. This experiment shows a clear and positive relationship between the collection of resin at the cambium and from the sapwood. For the purposes of this study, the benefit of being able to reseal the drilled holes after sampling was very beneficial to avoid attracting chemically-attuned beetles or other insects, and to minimize the damage to the phloem caused be frequent resampling.

Summary and Conclusions

This study set out to examine some of the mechanisms and physiology behind secondary mortality of ponderosa pines following prescribed fire. The experimental context consisted of a real life management challenge in the form of a fire restoration project in old growth ponderosa pine – mixed-conifer stands at Crater Lake NP, Oregon. The present study involved a multi-disciplinary investigation into various mechanisms behind bark beetle response to prescribed fire treatments, with an extensive review of existing literature as well as a number of experiments designed to answer relevant questions.

Various experiments confirmed that fire can increase resin flow in ponderosa pine. In the Crater Lake old-growth stand, resin flow appeared to peak in years 2 and 3 following fire, and to taper off by year 4 after fire treatment. The greatest effects were observed in more intense fall burn treatments compared with patchy spring burn treatments. In the parallel experiment in younger (50 year-old) trees at Sun Pass, changes to pre-burn resin flow were greatest one year after burning, and had returned to control levels by post-burn year three. Experiment 4 confirmed that most, or all of the observed post-fire resin flow increases could be explained by bole charring, rather than any effects on radial growth or other tree structures. The separate tree monitoring experiment at Crater Lake showed that post-fire mortality of the old trees matched treatment effects on resin flow patterns over time (higher mortality in higher resin flow treatments – fall burning and spring burning), undermining the conventional belief that resin defenses are indicative of survivorship. An investigation into fire effects on resin chemistry was mostly inconclusive. Finally, a methods investigation confirmed that resin volume collected by drilling into the sapwood is well correlated with resin collected at the outer xylem.

Integrating these findings with results from previous studies suggests that restoration effects on resin flow should be interpreted with caution. Resin flow measurements appear sensitive to both treatments that affect whole-tree vigor (constitutive resin) as well as to treatments that only cause bole injury. Historically, the effects of frequent low intensity fire probably contributed to both reduced competition (and higher vigor in surviving trees) and bole char injuries – both of which apparently can promote greater resin flow.

The importance of the complex mixture of airborne chemicals, including resin volatiles, on insect movements in forest ecosystems is only beginning to be understood. Although primary attraction in western pine beetles is not confirmed, monoterpene co-attraction (with beetle

pheromones) has long been known to occur. Primary attraction has also been observed many times in red turpentine beetles, which may have contributed to ponderosa pine mortality at Crater Lake, as well as in other bark beetles present in the area. Large monoterpene releases are known to occur following fire and other tree damage; continued ponderosa mortality (observed at Crater Lake) as well as heightened post-fire resin flow suggest that the potential benefits of increased resin defenses may be confounded by vast release of attractive resin compounds into the air.

The growth-differentiation balance was neither confirmed nor disproved in this study. The experiment on root and crown reduction may have been undermined by small sample sizes and uncertain treatment effects, but did not have any clear effects on resin flow. However, if we reject resin production and flow as the key determinants of tree resistance or susceptibility, then resource allocation tradeoffs seem secondary in determining post-fire mortality. A new conceptual model of fire-beetle interaction may be emerging based on both attractive and defensive roles of oleoresin. Ponderosa pine survival may be enhanced by minimizing local releases of resin volatiles, avoiding fire injury (or other types of tree damage), and promoting high constitutive resin production (for defenses in the case of attack).

Overall management implications can mostly be deduced from other reviews (Kolb et al. 2007 [see appendix 3 here], Seybold et al. 2006). Pine survivorship appears most closely related to high radial growth/BAI and high crown vigor. Resin flow monitoring in ponderosa pine is not a reliable indicator of the likelihood of survival after fire. Finally, although it remains untested, there may be merit to minimizing treatments that release resin volatiles. This includes avoiding injuring trees of interest, ensuring that thinning treatments include off-site disposal of thinned biomass, and an incremental approach to fire restoration, with repeated low intensity burns potentially more desirable than fewer higher intensity burns.

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Appendix 1. Latin and common names of species used in text

Common name	Latin name
Bark beetle	Coleoptera (Scolytidae)
Western pine beetle	*Dendroctonus brevicomis*
Mountain pine beetle	*D. ponderosae*
Red turpentine beetle	*D. valens*
Ips species	*Ips spp.*
Ponderosa pine	*Pinus ponderosa*
Loblolly pine	*P. taeda*
Lodgepole pine	*P. contorta*
Western white pine	*P. monticola*
Sugar pine	*P. lambertiana*
Eastern white pine	*P. strobus*
Aleppo pine	*P. halepensis*
Red pine	*P. resinosa*
White fir	*Abies concolor*
Grand fir	*A. grandis*
Norway spruce	*Picea abies*
Larch species	*Larix spp.*
Douglas-fir	*Pseudotsuga menziesii*
Trembling aspen	*Populus tremuloides*
Blue-stain fungus	*Ceratocystis* sp.

Appendix 2. Perrakis, D. D. B., and J. K. Agee. 2006. Seasonal fire effects on mixed-conifer forest structure and ponderosa pine resin properties. Canadian Journal of Forest Research **36**:238-254.

Appendix 3. Kolb, T. E., J. K. Agee, P. Z. Fule, N. G. McDowell, K. Pearson, A. Sala, and R. H. Waring. 2007. Perpetuating old ponderosa pine. Forest Ecology and Management **249**:141-157.