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EFFECTS OF HOST STORED RESOURCES ON BARK BEETLE-FUNGAL-CONIFER INTERACTIONS

By

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ABSTRACT

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Effects of host stored resources on bark beetle-fungal-conifer interactions

Chairperson: Dr. Anna Sala

Bark beetles and their associated fungi are among the greatest natural threats to conifers worldwide, but the degree to which host stored resources influence tree-beetle-fungal interactions has not been investigated. In western North America, the range of the mountain pine beetle (*Dendroctonus ponderosae)* has expanded from lower elevation *Pinus contorta* (lodgepole pine) forests into high elevation *Pinus albicaulis* (whitebark pine), a presumed superior host. I investigated whether stored resources in tree sapwood change after *D. ponderosae* attack, and whether this change relates to fungal colonization and beetle performance. I also studied how phloem and sapwood resources vary with elevation and tree diameter and examined the effect of tree species and diameter on *D. ponderosae* host selection.

Following beetle attack and fungal colonization, sapwood non-structural carbohydrates (NSC), lipids, and phosphorus declined in attacked trees relative to un-attacked trees. Resource declines were related to the degree of fungal colonization, suggesting a direct benefit to fungi in both host species. In *P. contorta*, beetle performance was also positively related to stored resources. The concentration of stored resources was generally higher in *P. albicaulis* than in *P. contorta* and increased with elevation and tree diameter, suggesting a potential increase in host quality for *D. ponderosae* and/or fungi. Beetles preferred larger diameter hosts, and although stored resources did not affect beetle performance in *P. albicaulis*, beetles were more likely to attack *P. albicaulis* even when larger *P. contorta* were available.

In a parallel system in Norway, phloem NSC and sapwood lipids also declined in *Picea abies* trees inoculated with the fungus *Ceratocystis polonica* relative to trees attacked by the bark beetle *Ips typographus* (which vectors *C. polonica*) or control trees, again indicating that stored resources enhance fungal colonization.

Overall, my results suggest that host stored resources influence the interaction between bark beetles, fungi, and conifers primarily by enhancing fungal growth. Fungal access to stored resources may also benefit beetles in some host tree species. A better understanding of the trophic interactions between beetles, fungi, and conifers may improve our ability to predict bark beetle dynamics and range expansion.

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PREFACE

Resource storage varies greatly in terrestrial plants (McGroddy 2004), causing variation in host quality for insect herbivores (Mattson 1980, Awmack and Leather 2002). In turn, insects have many strategies for maximizing nutrient uptake, which include selecting the highest quality hosts or engaging in interspecific mutualisms to obtain limiting nutrients (Six 2003, Mueller et al. 2005, Behmer 2009). Bark beetles (Curculionidae: Scolytinae) are highly successful forest insect herbivores that depend on low quality wood tissue, and use both of the above strategies to support large population outbreaks. The ultimate cause of bark beetle-fungal mutualisms is currently under debate (reviewed by Six and Wingfield 2011) because in some systems, bark beetles consume fungal hyphae to obtain nutrients and sterols (Barras 1973, Ayres et al. 2001, Bentz and Six 2006, Bleiker and Six 2007), and in other systems, fungal phytopathogenicity is thought to be critical in helping beetles overcome tree defenses (Raffa and Berryman 1982, Krokene et al. *in press*). Independent of the mechanism underlying beetle-fungal mutualisms, tree stored resources and resource utilization by fungi may indirectly benefit bark beetles if fungi provide important dietary benefits or if fungal growth has negative effects on the host tree.

In North America, fungal-derived dietary benefits are thought to be very important to the mountain pine beetle (*Dendroctonus ponderosae*), an aggressive beetle that is currently experiencing unprecedented population outbreaks (Logan and Powell 2001, Raffa et al. 2008). Given the potential importance of fungal-derived nutrients in this bark beetle-fungal relationship, there has been surprisingly little research on how tree stored resources influence the interaction between beetles and fungi. Mountain pine beetles are also experiencing range expansion from lower elevation lodgepole pine (*Pinus contorta*) forests into high elevation forests where the keystone species whitebark pine (*Pinus albicaulis*) occurs (Logan and Powell 2001, Raffa et al.

2008). A number of studies have investigated how beetles select lodgepole pine hosts, but less is known regarding beetle host selection in whitebark pine, or host selection when tree species co-occur. Whitebark pine could be a higher quality host than lodgepole pine, and if so, in addition to the increased threat to this important species, a higher quality host could influence the rate or extent of mountain pine beetle outbreaks.

In contrast to the mountain pine beetle, the European spruce bark beetle (*Ips typographus*) is often associated with a fungus that is thought to help beetles overcome Norway spruce (*Picea abies*) defenses (Krokene et al. 1999). The spruce bark beetle spends a shorter amount of time in its host tree than does the mountain pine beetle, which could inherently limit the ability of symbiotic fungi to redistribute tree resources in time to provide dietary benefits. However, tree stored resources and resource dynamics may still influence fungal performance, with consequences for the host tree (Ballard et al. 1983) and for spruce bark beetle outbreaks.

In this dissertation I examine how host stored resources influence the interaction between bark beetles, fungi, and conifers. Chapters 1-3 focus on the mountain pine beetle and two of its pine hosts: whitebark pine (*P. albicaulis*) and lodgepole pine (*P. contorta*), in the northern Rocky Mountains of Montana and Idaho, U.S.A. Chapter 4 was conducted in southern Norway and focuses on the European spruce bark beetle, the beetle's main fungal associate in southern Norway, *Ceratocystis polonica*, and Norway spruce. Overall, a better understanding of the implications of conifer stored resources on the complex interaction between bark beetles and fungi may ultimately improve our ability to forecast future bark beetle outbreaks and dynamics.

Chapter 1 examines whether tree sapwood stored resources, a nutrient pool available to fungi but not to beetles, change following mountain pine beetle attack and fungal colonization of whitebark pines and lodgepole pines. I found that sapwood non-structural carbohydrates (NSC), lipids, and phosphorus significantly declined in attacked relative to un-attacked trees. Sapwood nitrogen increased, but trees with more fungal colonization gained less nitrogen than un-attacked trees or trees with less fungal colonization. Further, NSC and nitrogen were positively related to beetle performance in lodgepole pines, but not in whitebark pines. This suggests that sapwood resources enhance fungal growth, but that the nutritional benefits provided by mutualistic fungi to *D. ponderosae* (Six 2003, Bleiker and Six 2007) may depend on the host tree species and the co-evolutionary relationship between specific beetle, fungal, and conifer species.

Chapter 2 reconstructs mountain pine beetle outbreaks to examine tree species versus diameter as beetle host selection cues. I found that tree diameter predicted beetle attack better than tree species, but when tree species significantly predicted beetle attack, whitebark pines were more likely to be attacked than lodgepole pines. At two sites, I calculated that whitebark pines were as likely to be attacked as lodgepole pines that were 10.7 or 14.7 cm larger in diameter. These results clearly indicate mountain pine beetle preference for whitebark pine over lodgepole pine where the two species co-occur, and suggest that small diameter whitebark pine are important in allowing mountain pine beetle outbreaks to persist at high elevation.

Chapter 3 examines the influence of elevation, species, and diameter on tree stored resources. I measured phloem and sapwood NSC, lipids, nitrogen, and phosphorus and found that, for both whitebark pines and lodgepole pines, resource concentrations increased with elevation and tree diameter. Resource storage was also generally higher in whitebark pines, where even small diameter trees had high resource concentrations relative to lodgepole pines. If whitebark pine becomes a common host for the mountain pine beetle in the future, and mutualistic fungi evolve to provide dietary benefits in this tree species (e.g. Ayres et al. 2001, Bleiker and Six 2007), my data suggest that large, high elevation whitebark pines may be a superior host for *D. ponderosae* because of their relatively greater concentrations of stored carbon compounds and mineral nutrients. This could have important management implications for high elevation ecosystems in the Rocky Mountains, where mountain pine beetle access to higher quality host trees may increase the extent of beetle outbreaks and threaten whitebark pine, an important keystone species.

Chapter 4 examines the relationship between tree resource dynamics and susceptibility to the phytopathogenic fungus *Ceratocystis polonica*, which is often vectored by the European spruce bark beetle *Ips typographus*. I found that phloem NSC and sapwood lipids declined in fungal inoculated Norway spruce (*Picea abies*) relative to beetle-attacked and control trees, and that negative correlations occurred between tree susceptibility and declines in nitrogen, NSC, and lipids over time. This suggests that stored resources benefit fungal growth with potential negative implications for host performance, including allocation to potent inducible defenses (Franceschi et al. 2005), and interference with water transport (Ballard et al. 1983).

Overall, my research demonstrates that host stored resources play an important role in the interaction between bark beetles, fungi, and conifers. In particular, my results suggest that fungi benefit from sapwood resources, but the mechanism by which beetles may benefit is unclear, and may be system specific and dependent on the co-evolutionary relationship between specific beetles, fungi, and host tree species. Future research on the trophic and evolutionary interactions between bark beetles, fungi, and conifers can inform the current debate on the causes of the beetle-fungal mutualisms and assist researchers in predicting bark beetle population dynamics over time and within different host trees.

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CHAPTER 4

Figure 1. Conceptual model illustrating three possible changes in tree resources in relation to tree susceptibility to fungal colonization. (A) If stored resources are overall more beneficial to the tree than to the fungus, trees that have higher initial resource concentrations and resource depletion over time (a negative change in resources) will be less susceptible and more resistant to fungal colonization. (B) Tree susceptibility may be independent of initial resource concentrations and tree susceptibility may cause complex changes in tree resources over time. For example, independent of initial resource concentrations, less susceptible trees may have more resource depletion and successfully resist fungal colonization (dashed line). Alternatively, independent of initial resource concentrations,

more susceptible trees may have more resource depletion because they are less resistant to fungal colonization and fungi consume resources (solid line). (C) If stored resources are overall more beneficial to the fungus than to the tree, trees with higher initial resource concentrations and resource depletion over time will be more susceptible and less resistant to fungal colonization. This could occur if high initial resource concentrations benefit fungi regardless of tree resistance, if resource consumption enhances fungal growth to such an extent that tree resistance breaks down, or if fungal resource consumption prevents tree resistance.

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CHAPTER 1

DO SAPWOOD STORED RESOURCES INFLUENCE THE INTERACTION BETWEEN MOUNTAIN PINE BEETLES, FUNGI, AND HIGH ELEVATION PINES?

Abstract Trophic interactions and their effects on community dynamics have long been a central topic in ecological research. Recent ecologically and economically destructive mountain pine beetle (*Dendroctonus ponderosae*) outbreaks have generated interest in understanding trophic interactions between beetles, symbiotic fungi (*Grosmannia clavigera* and *Ophiostoma montium*) and pine hosts. We asked whether tree sapwood stored resources, a nutrient pool available to fungi but not to beetles, influence the relationship between beetles and fungi. We observed that sapwood non-structural carbohydrates (NSC), lipids, and phosphorus significantly declined following beetle attack and fungal colonization of whitebark and lodgepole pine. We suggest that these sapwood nutrients enhance fungal growth, potentially minimizing resource competition between fungi and beetles in the phloem and promoting a mutualistic relationship between these organisms. Sapwood nutrients were also positively related to mountain pine beetle performance in lodgepole pine, its co-evolved host, but not in whitebark pine, a historically uncommon host tree, despite the higher nutritional quality of whitebark pine sapwood. This suggests that some of the nutritional benefits of the beetle-fungal mutualism may break down in the novel host tree. A better understanding of resource partitioning in this tri-trophic interaction informs the current debate on the proximate versus ultimate cause of the widespread relationship between bark beetles and fungi, provides insight into how two mutualists divide resources when part of their life cycle is spent in competition, and assists us in understanding mountain pine beetle outbreak dynamics over time and within different host trees.

Keywords *Pinus albicaulis*, *Pinus contorta*, non-structural carbohydrate, sapwood, trophic interactions

Introduction

A central idea in ecology is that communities and ecosystems are influenced by complex trophic interactions between organisms (e.g., Paine 1966; Pimm 1982; Polis 1991; Polis and Strong 1996; Schmitz *et al.* 2000). Understanding population dynamics and community structure depends on understanding energy and nutrient transfer between trophic levels. Organisms use a variety of methods to maximize nutrient uptake, and insects exemplify this variety with a range of strategies that include behavioral or physiological adjustments (Mattson 1980; White 1993; Simpson and Raubenheimer 2001; Raubenheimer and Simpson 2004; Frost 2005) and inter-species mutualisms (Higashi et al. 199; Bignell 2000; Six and Klepzig 2004; Schultz 2005; McCutcheon et al. 2009). In particular, mutualisms with bacteria or fungi can help insect herbivores maximize their nutrient uptake by allowing them to access plant tissue that is unreachable or indigestible.

Terrestrial plants vary tremendously in the nutrient composition and quality of their tissues (Hessen et al. 2004; McGroddy 2004). Abundant tissues like wood are generally of the lowest nutritional quality, and many insects that specialize on wood generally do so with the help of bacterial or fungal mutualists (Bignell 2000; Six 2003). For example, termites obtain limiting nutrients via partnerships with gut protozoa and bacteria, or by cultivating fungal gardens (Cleveland 1924; Higashi et al. 1992; Bignell 2000; Aanen and Boomsma 2005). Ambrosia beetles living in the wood of trees cultivate fungi (Farrell et al. 2001; Mueller et al. 2005), and bark beetles, which are closely related to ambrosia beetles but live in tree phloem, are also associated with symbiotic fungi (Six and Paine 1998; Six and Klepzig 2004). Trophic interactions between bark beetles, fungi, and trees have been the subject of a great deal of debate, yet remain poorly understood despite the economic and ecological importance of bark beetle outbreaks and recent range expansion of the mountain pine beetle (Logan and Powell 2001; Raffa et al. 2008).

In the past, fungi associated with bark beetles were thought to help beetles overcome tree defenses (Raffa and Berryman 1982; Christiansen and Solheim 1990; Solheim and Krokene 1998; Krokene et al. 1999; Lieutier et al. 2009), but recent work suggests that some fungi provide beetles with sterols (Bentz and Six 2006) or important nutrients (reviewed by Six 2003; Six and Klepzig 2004; Six and Wingfield 2010). Studies of the southern pine beetle (*Dendroctonus frontalis*) and the mountain pine beetle (*Dendroctonus ponderosae*) show that symbiotic fungi increase nitrogen in tree phloem, thereby producing a more favorable environment for developing beetle larvae (Ayres et al. 2000; Bleiker and Six 2007; Cook et al. 2010). Beetles that consume symbiotic fungi can also develop faster and produce larger broods than beetles that do not possess fungal symbionts (Six and Paine 1998; Ayres et al. 2000; Adams and Six 2007; Bleiker and Six 2007).

Past studies of beetle performance have focused on the nutritional quality and thickness of tree phloem, where bark beetles develop. However, unlike beetles, fungi grow deep into the sapwood of the tree, and it is not known whether sapwood stored resources may also influence the interaction between beetles and fungi. Although sapwood nutrient concentrations are low, total pools may be high due to the large volume of sapwood in a tree. Not only are fungi efficient at extracting nutrients from low quality substrate, but fungi can transfer nutrients from sources to sinks (Marler et al. 1999; Carey et al. 2004), and a continuous fungal connection between the sapwood and phloem could shuttle resources from the sapwood into the phloem where bark beetles develop. This may be particularly relevant in the relationship between the mountain pine beetle and its symbiotic fungi. Recently, beetle range has expanded from lower elevation lodgepole pine to higher elevation whitebark pine in the northern Rocky Mountains, and sapwood resource storage is expected to be higher in whitebark pine (further described below). A better understanding of the role of sapwood nutrients in the interaction between beetles, fungi and host tree species could therefore prove important in understanding subsequent effects on beetle performance and population dynamics.

Here we test the hypothesis that stored resources in tree sapwood benefit the mountain pine beetle and its symbiotic fungi. Specifically, we hypothesize that trees with initially more sapwood nutrients will support higher levels of fungal growth and more or better conditioned beetles. Alternatively, we hypothesize that sapwood nutrients are exploited by fungi but not transferred to beetles. If this is the case, we predict that sapwood nutrients will decline following beetle attack and fungal colonization, but beetle performance will not correlate with change in nutrient levels or fungal abundance. The following specific predictions were tested: (1) Whitebark pine (*Pinus albicaulis*) will have higher levels of sapwood resources (non-structural carbohydrates, lipids, nitrogen, and phosphorus) than lodgepole pine (*Pinus contorta*); (2) Beetles will preferentially attack trees with higher initial levels of sapwood resources; (3) Sapwood resources will decline following beetle attack and fungal growth; (4) The degree of fungal growth in the sapwood will positively correlate with sapwood resource change; (5) Trees with higher sapwood resources will produce more beetles or beetles with better body condition than trees with lower levels of sapwood resources.

Methods

Study system

Whitebark pine (*Pinus albicaulis* Engelmann) is a keystone species occurring in the northern Rocky, Cascade, and Sierra Nevada Mountains. In Montana, it occurs from 2000-3500 m elevation. Lodgepole pine (*Pinus contorta* Douglas ex. Louden) occurs throughout the western United States and Canada and is the most common host tree of the mountain pine beetle (Coulson and Witter 1984). In Montana, it occurs from 1000-3000 m elevation and frequently overlaps with whitebark pine at the edge of its range. Important differences in life history exist between these species, which could influence their relative nutrient storage in the sapwood. Whitebark pine is a longer lived species than lodgepole pine (Loehle 1988) and longevity in pines is related to slow growth rates due to higher investment in durability and stress resistance relative to biomass production (Loehle 1988, 1996; Larson 2001; Black et al. 2008). Further, lodgepole pine may retain needles for up to 18 years or more (Schoettle and Fahey 1994) while, based on our observations, whitebark pine retains needles only up to 8-10 years (A. Sala, unpublished data). Although comparative data on allocation to leaf area in lodgepole and whitebark pine at similar sites are not available, higher needle retention in lodgepole pine suggests higher relative allocation of resources to the foliage. If so, resource storage in the sapwood, particularly of non-structural carbohydrates and lipids, is expected to be lower in lodgepole relative to whitebark pine when growing at the same site.

The mountain pine beetle (*Dendroctonus ponderosae*) is the most aggressive and economically important bark beetle in North America (Coulson and Witter 1984; Paine et al.

1997; Raffa et al. 2008). Recent outbreaks have been attributed to warmer temperatures that improve survival, reduce development time, and allow the beetle to expand its range northwards and upwards in elevation (Bentz et al. 1991; Logan and Powell 2001; Carroll and Safranyik 2003). The mountain pine beetle is generally univoltine; larvae overwinter in the tree and emerge as adults in late summer. Dispersing adult beetles kill new trees in a pheromone-mediated mass attack that overcomes tree physical and chemical defenses (Wood 1982). Larval growth is aided by the ophiostomatoid fungi *Grosmannia clavigera,* considered a strong mutualist, and *Ophiostoma montium,* a weak mutualist (Six and Paine 1998; Six and Wingfield 2011). Adult beetles carry fungal spores in specialized mycangia and deposit one or both fungal species in the tree as they excavate egg laying galleries (Whitney and Farris 1970; Six and Paine 1999; Adams and Six 2007). Fungi colonize tree phloem and sapwood over the following weeks to months. Beetle larvae feed on both fungal hyphae and phloem tissue and new adults feed on fungal spores before emergence.

Study sites and field sampling

The study took place between 2008 and 2010. Study sites were located in the Pioneer and Absaroka-Beartooth Mountains of Montana. Vipond Park, in the Pioneer Mountains, consisted of an open canopy forest at 2500 m elevation (45°42’03.08” N, 112°55’41.98” W). Whitebark pine was the dominant tree at this site, followed by lodgepole pine, which occurred at low abundance. Very little understory existed at this site. Total annual precipitation was 213 mm in 2008, 253 mm in 2009, and 330 mm in 2010. Mean daily minimum and maximum air temperatures ranged from -15 °C to 0 °C in January and from 7 °C to 27 °C in July. Palmer Creek, in the Absaroka-

Beartooth Mountains, consisted of an open canopy mixed whitebark pine-lodgepole pine forest at 2667 m elevation, with a small subalpine fir understory (45°05’31.60” N, 110°58’58.58” W). Total annual precipitation was 238 mm in 2008, 340 mm in 2009, and 609 mm in 2010. Mean air temperatures ranged from -8 °C to 4 °C in January and from 10 °C to 29 °C in July. Healthy trees at each site were selected for initial sampling based on the position of trees attacked by the mountain pine beetle the previous summer. From our extensive surveys in 2007 and 2008, mountain pine beetle pressure at each site was judged to be moderate but increasing.

Beginning in summer 2008 (mid June-mid July), we obtained sapwood samples from approximately 60 whitebark pine and as many lodgepole pine trees as possible within the beetle attack front at each site. Diameter of all sampled trees ranged between 25 and 46 cm. We re-sampled a subset of un-attacked and naturally attacked trees eight weeks after mountain pine beetle attack (mid September-mid October), and again in summer 2009 before beetle emergence (mid June-mid July). This design enabled us to measure pre and post attack sapwood resources in as many naturally attacked trees as possible, excluding trees with unsuccessful beetle attacks. Sample sizes and characteristics of sampled trees, including mean diameter at breast height (DBH, measured 1.4 m above ground) and sapwood depth (in cm) are shown in Table 1. Sapwood depth, a linear measurement of the amount of water conducting xylem, is reported for comparison with tree diameter and depth of fungal bluestain. At each date, 1-3 wood cores per tree were obtained at breast height using a 5 mm hand increment borer. Sapwood for each tree was pooled, transported to the lab on ice, and placed in a 75 ºC drying oven for 48 hours. Samples were ground to powder using a Wylie Mill with a size 40 screen (General Electric) followed by a Genogrinder 2000 (OPS Diagnostics). This sampling design was repeated with a new set of approximately 50 whitebark pine and 25 lodgepole pine trees in 2009-2010.

Mountain pine beetle attack and emergence holes were counted in a 40 × 60 cm area on the north and south side of attacked trees at Palmer Creek for 2009-2010. Beetles were collected from attacked trees using one 40x60 cm mesh emergence trap on the south side of each tree (Bentz 2006), supplemented by hand collection from the tree. Beetle fat content, a measure of body condition, was determined using a petroleum ether extraction in a Soxhlet extractor (Kontes model 585050, Sigma-Aldrich, St. Louis, U.S.A.), according to Elkin and Reid (2004). The degree of fungal colonization of the sapwood was assessed by measuring the depth of bluestain in each wood core (in mm), starting from the bark where fungi were introduced, and measuring towards the center of the tree. Bluestain, caused by the melanization of fungal hyphae, occurs in both fungal species associated with the mountain pine beetle as well as in many other beetle-associated fungi (Paine et al. 1997; Klepzig 2005). Although bluestain does not always occur evenly in wood tissue that has been colonized by fungi, or may not occur immediately after colonization, measuring the depth of bluestain eight weeks and one year post-attack provided an estimate of the degree of fungal penetration into the sapwood of the tree at each time point.

Biochemical analyses

The sapwood was analyzed for non-structural carbohydrates (glucose, fructose, sucrose, and starch), lipids (acylglycerols), nitrogen, and phosphorus. Non-structural carbohydrates (NSC) were analyzed according to the photometric method of Hoch et al. (2002). Briefly, 12-14 mg of wood powder was extracted in 1.6 mL distilled water at 100 °C for one hour. An aliquot of this water was used to determine low molecular weight carbohydrates following enzymatic breakdown of fructose and sucrose to glucose. Following enzymatic breakdown of starch to glucose by a crude fungal amylase (‘Clarase’) at 40 °C overnight, and the conversion of glucose to gluconate-6-phosphate, the total glucose concentration was determined in a 96-well microplate reader at 340 nm. Sapwood lipids were analyzed using a similar photometric analysis according to Hoch et al. (1999). Briefly, 10-14 mg of wood powder was extracted in 1 mL aqueous NaOH for 30 minutes and glycerol was converted to glycerol-3-phosphate. The amount of liberated glycerol was determined in a 96-well microplate reader. Sapwood nitrogen content was measured by the University of California Davis Stable Isotope Facility, and phosphorus content was measured by the Colorado State University Soil Water and Plant Testing Laboratory.

Statistical analysis

A stepwise logistic regression using data from 2009 was used to determine whether species, site, tree morphological characteristics (DBH and sapwood depth) or initial sapwood resources (pre attack levels of NSC, lipids, nitrogen and phosphorus) predicted beetle attack (Predictions 1 & 2). After running this overall model, analyses were performed separately for whitebark pine for each site and year and lodgepole pine for each site in 2009. This and following analyses exclude lodgepole pine data for 2008, due to a small sample size, and also exclude sapwood depth in 2008 and sapwood phosphorus in both years at Vipond Park due to lack of measurements. Linear mixed models for 2008-2009 and 2009-2010 for each type of sapwood resource were used to determine whether a significant resource decline occurred following beetle attack and fungal colonization (Prediction 3). Models for 2009-2010 included beetle attack (attacked vs. un-attacked), tree species (whitebark pine vs. lodgepole pine), and an interaction between these as fixed factors, site (Palmer Creek vs. Vipond Park) as a random factor, and tree diameter as a covariate. Linear regressions were used to determine whether percent change in sapwood NSC, lipids, nitrogen, or phosphorus was related to tree species and degree of fungal colonization of the sapwood (Prediction 4). Data from Palmer Creek were analyzed separately for two intervals (zero to eight weeks or zero to one year post attack), for 2008-2009 and 2009-2010. Change in phosphorus was analyzed at one year post attack because phosphorus data were not collected at eight weeks. Data from Vipond Park were excluded from this analysis because of data structure concerns. Pearson correlations were used to compare beetle performance with tree morphological characteristics and sapwood resources at Palmer Creek, from 2009-2010 (Prediction 5). Beetle attack density was examined relative to tree DBH, sapwood depth, and initial resource levels. Beetle emergence density, relative emergence (the ratio of beetle emergence to beetle attack) and fat content were examined relative to tree resource levels at all time points, percent change in resources over time, and the degree of fungal colonization of the sapwood. Analyses were performed using PASW Statistics 18 (IBM Statistics).

Results

Tree morphological characteristics and sapwood resources varied between host tree species (Prediction 1, Figure 1). This variation had some effect on likelihood of mountain beetle attack, although contrary to Prediction 2, effects were not consistent between sites, species, or years (Table 2). An initial stepwise logistic regression including species, site, tree morphological characteristics (DBH and sapwood depth) and initial sapwood resources (pre attack levels of NSC, lipids, nitrogen and phosphorus) as predictors indicated that sapwood depth and DBH were significant predictors of likelihood of beetle attack ($\chi^2 = 6.858$, $p = 0.024$; $\chi^2 = 6.275$, $p = 0.034$, respectively). When we performed separate regressions for each species and site in each year (excluding 2008 lodgepole pine), we found no significant predictors of beetle attack in whitebark pine at Palmer Creek in 2008 or 2009 or at Vipond Park in 2008. Model results from 2008 are therefore not shown. In 2009, NSC, lipids, nitrogen, and sapwood depth had varying influence on the likelihood of beetle attack for each species at Vipond Park and for lodgepole pine at Palmer Creek; of particular note is that lodgepole pines with higher sapwood nitrogen were more likely to be attacked by the mountain pine beetle at Palmer Creek in 2009 (Table 2).

Significant changes in sapwood nutrients occurred one year following mountain pine beetle attack and fungal colonization (Prediction 3, Tables 3 & 4). Our linear mixed models for 2008-2009 show significant declines in sapwood NSC and lipids in attacked relative to un-attacked whitebark pine, but no change in sapwood nitrogen or phosphorus (Table 3). Models for 2009-2010 show that by one year post beetle attack, a significant decline in sapwood NSC and phosphorus occurred in attacked trees relative to un-attacked trees (Table 4). Sapwood lipids were influenced by site and a species × attack interaction, and at both sites a greater decline in sapwood lipids occurred in whitebark pine relative to lodgepole pine. Sapwood nitrogen was also influenced by site and by a species × attack interaction, but nitrogen increased in un-attacked and attacked trees over time, although for lodgepole pine at Palmer Creek and for both species at Vipond Park, attacked trees gained less nitrogen than un-attacked trees. To insure that a decline in nitrogen was not masked by changes in NSC or lipids, we subtracted the mass due to these compounds and re-calculated total sapwood nitrogen; however, increased sapwood nitrogen was not related to changes in sapwood NSC and lipids.

In general, sapwood nutrients declined as fungal colonization of the sapwood increased (Prediction 4, Figure 2). In 2009, an initial short-term increase in sapwood NSC was followed by a long-term decline. This short-term increase occurred for both un-attacked and attacked trees, but attacked trees gained significantly less NSC relative to un-attacked trees. Sapwood lipids generally declined as fungal colonization increased, with the exception that in 2009 un-attacked trees also showed a long-term decline. Sapwood phosphorus declined in 2009 as fungal colonization increased ($R^2 = 0.279$, $p = 0.003$; since data were only available 1 year post-attack, this result is not shown in Figure 2). In contrast, sapwood nitrogen increased in the long term in both un-attacked and attacked trees, although in 2009, trees with a greater degree of sapwood fungal colonization gained less nitrogen.

Mountain pine beetle performance correlated with some tree morphological characteristics and some measures of sapwood resources, particularly in lodgepole pine (Prediction 5, Figure 3). Average beetle attack density did not differ between whitebark and lodgepole pine (40 ± 13 attacks m^{-2} in whitebark pine and 37 ± 22 m^{-2} in lodgepole pine). In whitebark pine, beetle attack density was positively correlated with sapwood depth ($R = 0.757$, $p = 0.011$, $N = 10$), but no correlations existed between attack density and initial tree resource levels. Average beetle emergence density also did not differ between whitebark and lodgepole pine (67 ± 24 emerged m^{-2} in whitebark pine and 52 ± 19 m^{-2} in lodgepole pine). Beetle emergence from whitebark pine was negatively correlated with initial sapwood phosphorus ($R = -0.668$, $p = 0.035$, $N = 10$). In lodgepole pine, attack density was not correlated with tree characteristics or sapwood resources. Beetle emergence was negatively correlated with initial sapwood NSC ($R = -0.778$, $p = 0.023$, $N = 8$; Figure 3a), and positively correlated with sapwood NSC eight weeks following attack ($R = 0.712$, $p = 0.047$, $N = 8$; Figure 3b) and with the percent change in sapwood NSC that occurred over this time period ($R = 0.821$, $p = 0.012$, $N = 8$; Figure 3c). To account for attack density, we evaluated relative beetle emergence as the ratio of beetle emergence m^{-2} to beetle attack m^{-2}. In whitebark pine, relative beetle emergence did not correlate with any sapwood resources. In lodgepole pine, relative beetle emergence was positively correlated with NSC eight weeks following attack ($R = 0.858$, $p = 0.006$, $N = 8$; Figure 3f), with the percent change in NSC over this time period ($R = 0.850$, $p = 0.008$; $N = 8$; Figure 3g), and with initial sapwood nitrogen ($R = 0.732$, $p = 0.039$, $N = 8$; Figure 3h). Beetle fat content at emergence and fungal colonization of the sapwood were not correlated with any variable.

Discussion

Our results support our overall hypothesis that stored resources in tree sapwood are important in the interaction between the mountain pine beetle and its symbiotic fungi. Specifically, we found that sapwood nutrients declined dramatically following beetle attack and fungal colonization, that decline of non-structural carbohydrates and lipids was particularly associated with fungal growth, and that sapwood nutrients were positively related to beetle performance in lodgepole pine, the most common host, but not in whitebark pine, a less common host. Although we could not directly test whether fungi transfer nutrients from the sapwood, which is inaccessible to beetles, to the phloem, our results suggest that not only do sapwood stored resources influence the interaction between beetles and fungi, but that beetle success may be influenced by co-evolutionary history with different host tree species. Recent work has emphasized resource-driven hypotheses for the close relationship between beetles and fungi (reviewed by Six and Wingfield 2011), but the significance of a large nutrient pool in the sapwood and its consequences for fungal and beetle performance have historically been overlooked and may greatly benefit our understanding of the role of the host tree in this tri-trophic interaction.

As hypothesized, sapwood nutrient concentration was higher in whitebark pine relative to lodgepole pine (Figure 1). However, this did not translate to higher beetle attack in whitebark pine, suggesting that sapwood nutritional quality alone does not influence host preference. Further, tree characteristics such as diameter and sapwood depth (an indicator of sapwood volume and the total resource pool) were not consistent predictors of mountain pine beetle attack in either tree species. We did observe that beetle attack was more likely in lodgepole pine with higher sapwood nitrogen (Table 2). The underlying mechanism for this preference is unknown, but it may be related to the co-evolutionary history between the mountain pine beetle and lodgepole pine. Lodgepole pine is of lower nutritional quality, but the long co-evolutionary history between the mountain pine beetle and this tree may allow beetles to respond to choice cues that apparently do not exist for less common host trees like whitebark pine.

Following mountain pine beetle attack and fungal colonization, we generally observed a significant decline in sapwood non-structural carbohydrates (NSC), lipids, and phosphorus in both tree species (Tables 3 & 4). At Palmer Creek, the depletion of sapwood NSC and lipids resulted in an almost complete elimination of the mobile carbon pool. Such an extreme depletion is highly unusual; trees contain enough stored mobile carbon in the sapwood to re-foliate themselves multiple times (Li et al. 2002; Hoch et al. 2003). Even during natural events such as drought, trees may die long before their mobile carbon reserves are exhausted to the degree we observed (Piper et al. 2009). Although a major long-term decrease in sapwood NSC and lipids occurred after beetle attack, NSC and lipids occasionally increased immediately following attack, likely reflecting that photosynthesis temporarily continued in some trees (Figure 2). In contrast, sapwood nitrogen tended to increase in the long term in both attacked and un-attacked trees, possibly related to changes in sapwood or cellular properties over time (Cowling and

Merrill 1966). While sapwood nitrogen did not decrease as a result of beetle attack or fungal colonization, attacked trees gained less nitrogen relative to un-attacked trees, and attacked trees with heavily colonized sapwood tended to gain less nitrogen than attacked trees where the sapwood was lightly colonized by fungi (Figure 2). Therefore, we suggest that sapwood nitrogen may still be used by fungi following mountain pine beetle attack, but perhaps not enough to offset environmental effects on nitrogen concentration and result in an absolute depletion.

Our results indicate that sapwood nutrient depletion is a result of fungal colonization and consumption. Because bark beetles develop entirely in the phloem of the tree, sapwood nutrient declines cannot be due to direct beetle consumption. Alternative explanations to fungal-driven depletion of sapwood nutrients seem unlikely. One alternative is that NSC and lipid depletion relates to the allocation of sapwood nutrients for defense against beetle attack. However, tree defenses are exhausted within days in a successful mountain pine beetle attack (Raffa and Berryman 1983; Paine et al. 1997), while sapwood NSC and lipids continued to decline between eight weeks and one year following beetle attack in our study. This suggests that the declines in sapwood NSC and lipids observed here are unrelated to tree defense. Sapwood resource depletion after beetle attack could also reflect allocation to the tree canopy. However, this is also unlikely; the girdling effect of beetles can impede phloem transport and the declines in sapwood resources that we observed were extremely large. Ongoing research further indicates that depletion of sapwood NSC and lipids following beetle attack is significantly greater than that following manual girdling of trees (E. Lahr unpublished data). Therefore, we suggest that depletion of sapwood resources is due to fungal consumption and directly benefits fungal growth. However, the subsequent effect of these nutrients on beetle performance was different between the common host tree (lodgepole pine) and the less common host, whitebark pine.

We saw no difference in beetle attack or emergence density between tree species, but we found that initial sapwood nitrogen and midseason levels of sapwood NSC had a strong positive correlation with beetle emergence from lodgepole pine (Figure 3). Given the well-known importance of phloem nutritional quality to beetle performance (Ayres et al. 2000; Bleiker and Six 2007), an immediate question is whether a positive effect of sapwood nutrients on beetle performance simply reflects higher phloem nutrients. In this case, a positive correlation between beetle performance and sapwood nutrients should have also been observed in whitebark pine, but it was not. Therefore, it appears that while sapwood nutrients had a positive effect on fungal growth in both host trees, a positive effect of sapwood nutrients on beetle performance occurred only in lodgepole pine, the common host. These results again suggest that the interaction between beetles, fungi, and trees reflects their co-evolutionary relationships. The strong negative correlation between beetle emergence and initial sapwood NSC, at first counterintuitive, may further reflect this relationship in lodgepole pine (Figure 3). The mountain pine beetle is attuned to variation in lodgepole pine defenses (Raffa and Berryman 1982, 1983), and lower initial sapwood NSC may reflect trees with lower defenses. If there is a trade-off between growth and defense, trees with lower defenses could also have greater growth potential and more phloem (Thomson and Shrimpton 1985; Lorio and Sommers 1986), further supporting the relationship between initial sapwood NSC and beetle performance in lodgepole pine.

Although we did not directly test whether fungi mediate nutrient transport from sapwood to beetles, the consumption of sapwood nutrients by fungi and the positive correlation between sapwood nutrients and beetle performance in lodgepole pine may be explained in the following way. Fungi benefit from their relationship with beetles by being transported to new host trees. Beetle larvae, in turn, benefit from fungal hyphae as an important food source throughout their development; fungi can concentrate nitrogen and phosphorus in the phloem (Ayres et al. 2000; Bleiker and Six 2007) and provide sterols (Bentz and Six 2006). Since fungi must sporulate in the phloem to receive beetle transport to a new tree, fungi must continuously replace the tissue eaten by beetle larvae. The sapwood may therefore provide a large pool of carbohydrates, lipids, and perhaps nitrogen that fungi can draw on to support growth in the phloem despite continual pressure imposed by beetles. If so, fungal growth sustained by sapwood nutrients can provide indirect benefits to the mountain pine beetle. Interestingly, this mechanism does not appear to fully operate in whitebark pine, where higher sapwood nutritional quality and fungal-driven depletion of sapwood nutrients did not translate into increased beetle performance relative to lodgepole pine. Fungi may still rely on sapwood nutrients in whitebark pine, but these results suggest that in terms of providing nutritional benefits to beetles, the relationship between beetles and fungi appears to break down in the less common host tree. Future research is necessary to determine whether fungal growth or ability to concentrate resources is host tree dependent and reflects the co-evolutionary relationship between beetles, fungi, and trees.

Overall, our study highlights that in order to properly understand the tri-trophic interaction between the mountain pine beetle, its symbiotic fungi and its tree hosts, we must understand the role of tree resource dynamics and sapwood nutrient storage. Now that our research has demonstrated the importance of tree sapwood nutrients, understanding whether beetles directly benefit from these resources via a fungal conduit from the sapwood to the phloem, and whether direct or indirect benefits to beetles vary depending on the host and on the specific fungal partner could assist in our understanding of mountain pine beetle productivity. The fungi *Grosmannia clavigera* and *Ophiostoma montium* have a mutualistic but asymmetric relationship with the mountain pine beetle (Six and Paine 1998; Six and Bentz 2007; Cook et al.

2010), and the apparent breakdown of the fungus-beetle mutualism in whitebark pine could be due to a shift in the presence or relative abundance of either fungal species, or to differences in their ability to partition or concentrate tree resources. During the year-long development of the mountain pine beetle, other fungi and bacteria may also begin to impact tree stored resources. A more detailed understanding of energy and nutrient transfer between organisms in this interaction can inform the current debate on the proximate versus ultimate cause of the widespread relationship between bark beetles and fungi and assist us in understanding mountain pine beetle outbreak dynamics over time and within different host trees.

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Table 1. Sample sizes and tree characteristics at each site in 2008 and 2009.

Year	Site	Species[a]	Description[b]	N[c]	Diameter (cm)[d]	Sapwood (cm)[e]
2008	Vipond Park	WBP	Healthy	12	27.0 (6.0)	-
		WBP	Attacked	13	28.0 (4.6)	-
		LPP	Healthy	3	32.1 (7.1)	-
		LPP	Attacked	2	31.9 (2.6)	-
	Palmer Creek	WBP	Healthy	8	39.5 (14.0)	4.0 (2.1)
		WBP	Attacked	11	45.8 (21.7)	4.0 (1.2)
		LPP	Healthy	6	38.1 (12.9)	5.8 (2.2)
		LPP	Attacked	2	52.7 (23.5)	4.8 (1.5)
2009	Vipond Park	WBP	Healthy	11	29.5 (6.8)	5.3 (1.7)
		WBP	Attacked	7	26.0 (4.1)	3.7 (0.9)
		LPP	Healthy	7	25.6 (7.7)	5.8 (1.0)
		LPP	Attacked	5	25.0 (5.2)	3.6 (1.2)
	Palmer Creek	WBP	Healthy	9	28.7 (4.0)	3.7 (1.2)
		WBP	Attacked	10	37.6 (13.4)	3.4 (1.4)
		LPP	Healthy	11	29.0 (4.6)	5.3 (1.1)
		LPP	Attacked	7	38.7 (11.2)	5.5 (1.4)

[a] Tree species are whitebark pine (WBP) and lodgepole pine (LPP).

[b] Trees remained healthy or were attacked by the mountain pine beetle following sampling.

[c] Mean tree diameter at breast height (standard deviation).

[d] Mean sapwood depth (standard deviation); not measured at Vipond Park in 2008.

Table 2. Stepwise forward logistic regression showing predictors of mountain pine beetle attack in 2009 for whitebark pine and lodgepole pine at Palmer Creek and Vipond Park.

		Palmer Creek		Vipond Park	
Predictor	Model Result	WBP	LPP	WBP	LPP
NSC	p-value (initial)	0.747	0.034	**0.050**	0.925
	p-value (model entry)	-	-	**0.050**	-
	χ2	-	-	**13.675**	-
	B	-	-	**-444.963**	-
Lipids	p-value (initial)	0.659	0.645	**0.370**	0.059
	p-value (model entry)	-	-	**0.009**	-
	χ2	-	-	**13.266**	-
	B	-	-	**-566.784**	-
Nitrogen	p-value (initial)	0.783	**0.006**	0.283	0.884
	p-value (model entry)	-	**0.006**	-	-
	χ2	-	**9.033**	-	-
	B	-	**18.761**	-	-
Phosphorus	p-value (initial)	0.815	0.020	-	-
Diameter	p-value (initial)	0.069	0.032	0.297	0.875
Sapwood	p-value (initial)	0.692	0.609	**0.072**	**0.012**
	p-value (model entry)	-	-	**0.037**	**0.012**
	χ2	-	-	**16.416**	**8.218**
	B	-	-	**-10.146**	**-0.210**

Predictors of mountain pine beetle attack in whitebark pine (WBP) and lodgepole pine (LPP) include pre attack levels of sapwood non-structural carbohydrates (NSC), lipids, nitrogen, phosphorus, tree diameter at breast height, and tree sapwood depth. The model results shown for each predictor include its initial significance (p-value), its significance at entry into the model (p-value), the Chi square test statistic change in -2 log likelihood (χ^2), and the regression coefficient (B). Positive values of B indicate that increases in the measured variable increase likelihood of attack, and negative values indicate a decreased likelihood of attack. Bolded results further emphasize the significant predictors of beetle attack, while un-bolded results were not included in the final model as predictors of beetle attack. Sample sizes were N = 19 for whitebark pine and N = 18 for lodgepole pine at Palmer Creek, N = 18 for whitebark pine and N = 12 for lodgepole pine at Vipond Park. No significant predictors of beetle attack occurred at either site in 2008.

Table 3. Linear mixed model showing percent change in sapwood resources for 2008-2009 for whitebark pine at Palmer Creek and Vipond Park.

	NSC			Lipids			Nitrogen			Phosphorus		
Variable	F	p	B	F	p	B	F	p	B	F	p	B
Intercept	0.065	0.802	-1.188	0.949	0.476	-0.676	2.865	0.250	0.194	0.468	0.504	0.330
Attack	13.644	**0.001**	1.462	7.148	**0.011**	0.213	0.218	0.643	-0.036	0.616	0.444	0.554
Site	1.679	0.202	0.598	36.685	**0.000**	0.558	16.815	**0.000**	0.371	-	-	-
Diameter	0.018	0.895	-0.002	2.271	0.140	-0.005	4.427	**0.042**	-0.006	0.021	0.888	-0.003

For each variable, the F-statistic (F), p-value (p) and regression coefficient (B) are shown. Degrees of freedom = 1, N = 44 for non-structural carbohydrates (NSC) and nitrogen. Degrees of freedom = 1, N = 41 for lipids. Degrees of freedom = 2, N = 19 for phosphorus. Attack was a fixed factor, site was a random factor, and diameter was a covariate. Phosphorus was not measured at Vipond Park. Bolded p-values further emphasize significant variables.

Table 4. Linear mixed model showing percent change in sapwood resources for 2009-2010 for whitebark pine and lodgepole pine at Palmer Creek and Vipond Park.

	NSC			Lipids			Nitrogen			Phosphorus		
Variable	F	p	B	F	p	B	F	p	B	F	p	B
Intercept	0.239	0.627	0.042	2.428	0.216	-0.543	6.673	0.104	0.271	48.612	0.000	-0.830
Attack	4.784	**0.033**	0.746	1.184	0.281	0.474	40.526	**0.000**	0.573	16.958	**0.000**	0.203
Species	2.59	0.113	-0.013	0.039	0.844	0.355	0.190	0.664	0.245	2.085	0.158	-0.133
Site	0.524	0.472	-0.151	16.372	**0.000**	-0.555	22.526	**0.000**	-0.271	-	-	-
Diameter	0.647	0.424	-0.01	0.188	0.666	0.003	0.126	0.723	0.001	0.502	0.483	0.002
Attack × Species	2.388	0.127	-0.607	6.481	**0.013**	-0.659	16.937	**0.000**	-0.442	0.907	0.348	0.106

For each variable, the F-statistic (F), p-value (p) and regression coefficient (B) are shown. Degrees of freedom = 1, N = 65 for non-structural carbohydrates (NSC), lipids, and nitrogen. Degrees of freedom = 4, N = 38 for phosphorus. Attack and species were fixed factors, site was a random factor, and diameter was a covariate. Phosphorus was not measured at Vipond Park. Bolded p-values further emphasize significant variables.

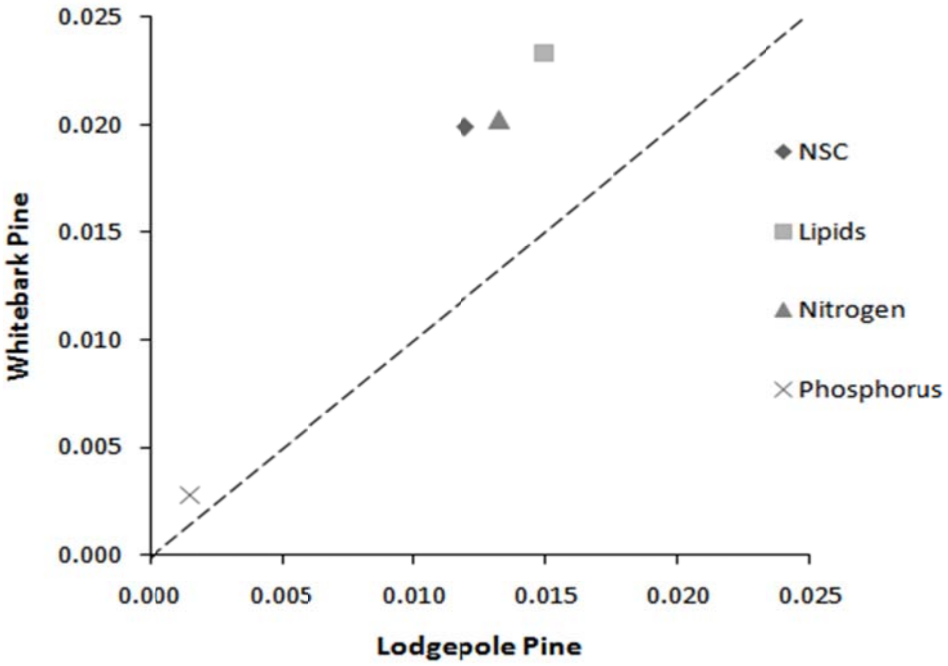

Figure 1. The ratio of sapwood resource levels in whitebark pine to those in lodgepole pine, prior to beetle attack. Data from 2009 were pooled for Palmer Creek and Vipond Park and standardized by sapwood depth (N = 37 whitebark pine and N = 30 lodgepole pine). The units for non-structural carbohydrates (NSC) and lipids are % dry weight, and the units for nitrogen and phosphorus are µg/mg. The dashed line represents 1:1.

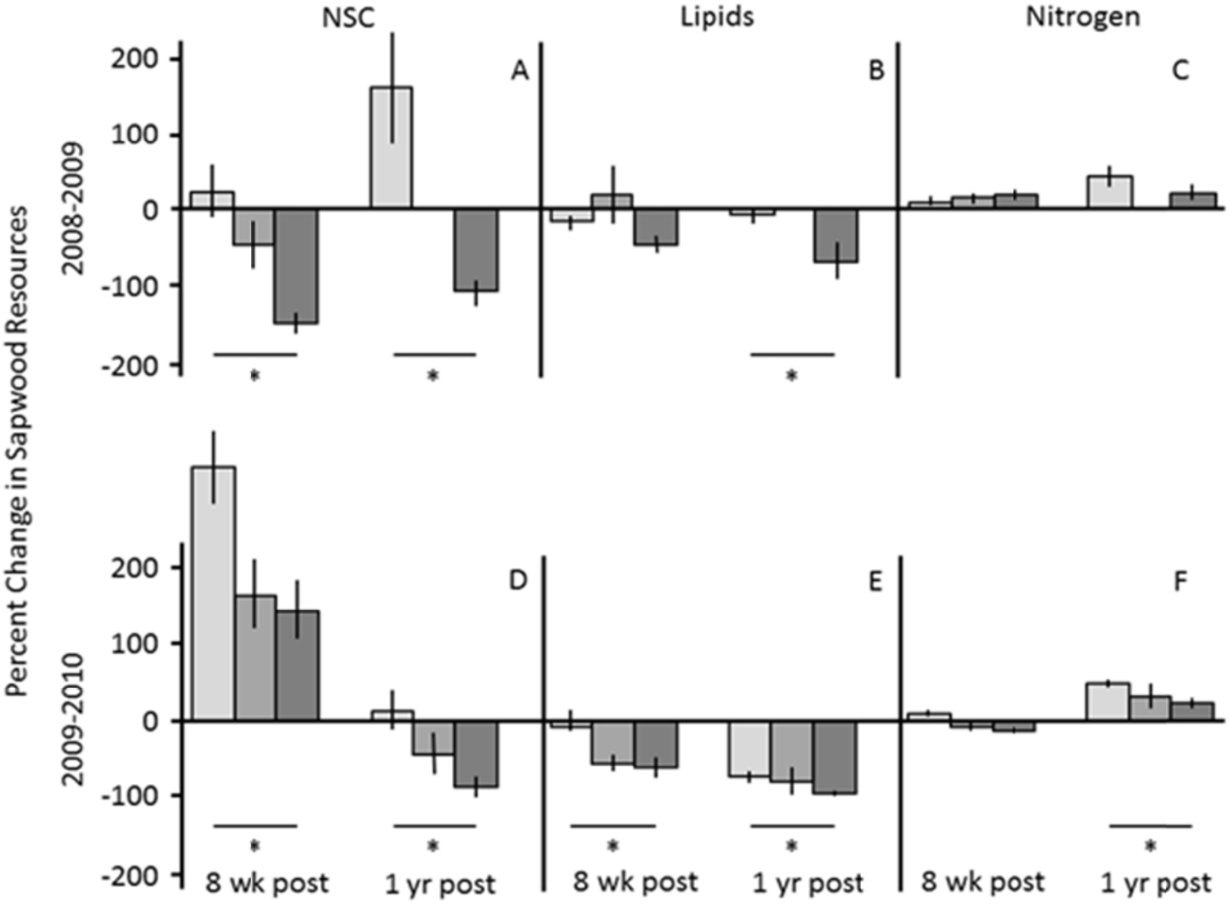

Figure 2. Percent change in sapwood resources following beetle attack in 2008-2009 (top) and 2009-2010 (bottom) in relation to fungal colonization. Non-structural carbohydrates (NSC) are shown in panels A and D, lipids in panels B and E, and nitrogen in panels C and F. Light gray bars represent un-attacked trees, medium gray bars represent attacked trees with less than 25% sapwood bluestain, and dark bars represent attacked trees with greater than 50% sapwood bluestain. For clarity, whitebark pine and lodgepole pine at Palmer Creek are pooled in this figure. Sample size for each year can be found in Table 1. Bars show mean ± 1 SEM. Asterisks under bars indicate a significant relationship (R^2 from 0.278 to 0.497 and $p < 0.05$) between degree of fungal colonization and percent change in sapwood resources, using linear regression.

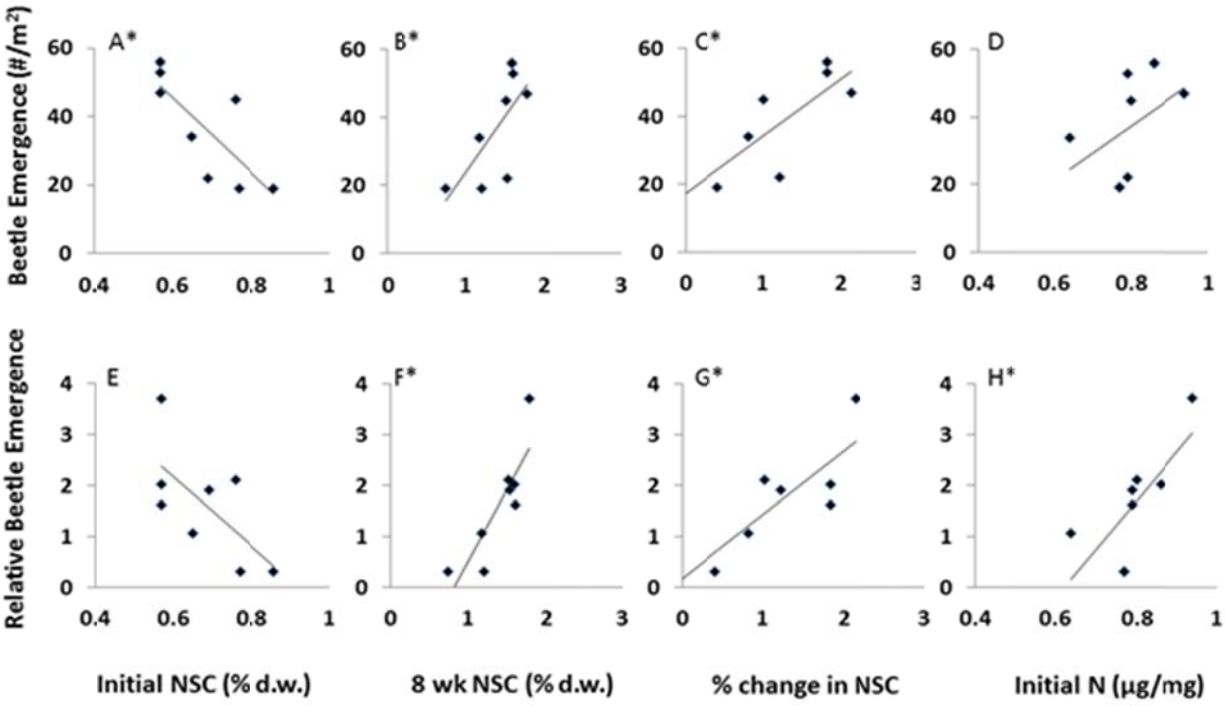

Figure 3. Beetle performance relative to sapwood resources in lodgepole pine. Panels A-D show beetle emergence (no. m^{-2}), and panels E-H show relative beetle emergence (the ratio of emergence m^{-2} to attack m^{-2}), relative to initial sapwood non-structural carbohydrates (NSC), sapwood NSC eight weeks following beetle attack, percent change in sapwood NSC from 0-8 weeks, and initial sapwood nitrogen (N). Linear trend lines are included in each panel. Asterisks next to figures letter indicate significant correlations, as described in the Results.

CHAPTER 2

DOES TREE SPECIES OR DIAMETER BETER PREDICT MOUNTAIN PINE BEETLE ATTACK WHERE WHITEBARK PINE AND LODGEPOLE PINE CO-OCCUR?

Abstract Mountain pine beetle (*Dendroctonus ponderosae*) outbreaks pose a significant threat to high elevation ecosystems in the Rocky Mountains. Of particular concern is beetle preference for whitebark pine over lower elevation lodgepole pine. We used a canopy foliage scoring system to reconstruct beetle outbreaks at three sites in western Montana, and to evaluate the importance of tree species versus diameter as host selection cues. We found that tree diameter predicted the probability of beetle attack better than tree species, but when tree species significantly predicted beetle attack, whitebark pine was more likely to be attacked than lodgepole pine. Although the largest diameter trees of each species were attacked first, generally more whitebark pines than lodgepole pines were attacked, and beetles attacked whitebark pines less than 20 cm in diameter even when larger lodgepole pines were available. At two sites, we calculated that whitebark pines were as likely to be attacked as lodgepole pines that were 10.7 or 14.7 cm larger in diameter. Our study documents beetle preference for whitebark pine over lodgepole pine where the two species co-occur, and indicates the importance of small diameter whitebark pine in allowing the persistence of mountain pine beetle outbreaks at high elevation.

Introduction

The mountain pine beetle (*Dendroctonus ponderosae*) is an aggressive bark beetle whose host selection behavior varies greatly during the course of a population outbreak (Alcock 1981; Raffa and Berryman 1983; Bentz et al. 1993; Raffa et al. 2008; Boone et al. 2011). Although it is a generalist on most *Pinus* species, the mountain pine beetle most commonly occurs in lodgepole pine (*Pinus contorta*) in the northern Rocky Mountains (Amman and Cole 1983; Coulson and Whitter 1984; Safranyik 2003). Endemic beetle populations are found in stressed or weakened trees, but during outbreaks, mountain pine beetles attack larger, more vigorous, and better defended trees (Cole et al. 1981; Raffa and Berryman 1982; Shrimpton and Thomson 1983; Waring and Pitman 1985; Bentz et al. 2005; Clark et al. 2010; Boone et al. 2011). Larger lodgepole pines generally have thicker phloem and are a better food source for the phloeophagous beetle larvae (Amman and Cole 1983; Amman and Pasek 1986; Zausen et al. 2005; Boone et al. 2011; but see Thomson and Shrimpton 1985).

In the past, mountain pine beetles rarely occurred at high elevations, but warmer winters and large outbreaks have increased beetle range dramatically in recent years (Logan and Powell 2001; Carroll and Safranyik 2003; Raffa et al. 2008). This allows the mountain pine beetle access to tree species that were historically unavailable, such as whitebark pine (*Pinus albicaulis*), a species that beetles may prefer over lodgepole pine (Baker et al. 1971; Amman 1982; Six and Adams 2007). Although tree diameter is an important cue that mountain pine beetles use to select hosts (Cole et al. 1969; Amman and Cole 1983), and lodgepole pine often exceeds whitebark pine in diameter where the two species co-occur, whitebark pine may have thicker phloem (Amman 1982; Six and Adams 2007; but see Baker et al. 1971) and higher resource concentrations in both phloem and sapwood (Amman 1982; Lahr and Sala in prep). Because the range of the mountain pine beetle is expanding, it is becoming more important to understand the factors that underlie host selection choices and to directly test predictions concerning the influence of tree species versus diameter on mountain pine beetle host selection.

It has been suggested that the mountain pine beetle may prefer whitebark pines over lodgepole pines where they co-occur (Baker et al. 1971; Six and Adams 2007; Dooley and Six unpublished data), which may have serious consequences for high elevation ecosystems in the Rocky Mountains. Whitebark pine is a keystone species that regulates snowmelt, facilitates plant succession, and provides a high energy food source for many sub-alpine animals. The combined effects of mountain pine beetles, an introduced fungal pathogen (*Cronartium ribicola*), and years of fire suppression may drive whitebark pine locally extinct (Tomback et al. 2001; Logan and Powell 2001). A better understanding of mountain pine beetle host selection could inform predictions regarding the rate and extent of high elevation beetle outbreaks and allow better evaluation of efforts to restore populations of whitebark pine.

Here we evaluate the extent to which tree species and tree diameter influence the probability of mountain pine beetle attack. We first reconstruct the progression of mountain pine beetle outbreaks in recently attacked stands containing both whitebark and lodgepole pine, and we determine the number and size of attacked trees relative to available trees in each year. We then ask, using statistical modeling, whether tree species or diameter predicts mountain pine beetle attack, and whether attack probabilities change over time as host species availability changes. We also calculate the change in tree diameter that produces the same probability of beetle attack as a change in tree species.

Methods

Data were collected at three sites in southwest Montana, U.S.A. during July – September 2008. Mt. Edith, in the Helena National Forest (46°24’33.07” N, 111°10’41.62” W, elevation 2286 m) suffered an intense mountain pine beetle outbreak that was rapidly declining at the time of this study. Vipond Park, in the Beaverhead-Deerlodge National Forest (45°42’03.08” N, 112°54’08.84” W, elevation 2280 m) and Palmer Creek, in the Gallatin National Forest (45°05’31.60” N, 110°58’58.58” W, elevation 2667 m) had moderate but increasing mountain pine beetle pressure. Stand composition was mixed at Mt. Edith and Vipond Park, and dominated by whitebark pine at Palmer Creek. At each site, lodgepole pines were generally larger. At each site, we established 4-6 adjacent 400 m^2 square plots, measured every tree in each plot, and pooled the plots for statistical analysis. Smaller plots rather than one large plot were used to make it easier to count and score all trees. The total study area encompassed 0.24 ha at Mt. Edith and Vipond Park, and 0.16 ha at Palmer Creek. The diameters of all whitebark pine and lodgepole pine trees were measured at breast height (1.4 m above ground), and un-attacked trees less than 10 cm in diameter were excluded from analyses.

At each site, we assessed mountain pine beetle preference at the tree level using a scoring system based on canopy needle color (Table 1). Trees were scored on a scale from 0-6, ranging from un-attacked with green needles (score of 0) to the oldest attacked trees with faded red needles and significant needle loss (score of 6). Trees with a score of 1 were attacked in 2008, the year this study occurred. Higher scores may roughly correspond to the date of beetle attack, but environmental variation could also influence canopy foliage differently at different sites (Wulder et al. 2006; Bockino 2007). Therefore, although we can confidently reconstruct the progression of an outbreak within a single site, we cannot say that a tree with a given score was attacked in the same year as a tree with the same score at a different site. In our statistical analyses, each canopy foliage score category reflects the actual number of available and attacked trees in that score.

We scored 172 whitebark and 102 lodgepole pines at Mt. Edith, 110 whitebark and 70 lodgepole pines at Vipond Park, and 172 whitebark and 19 lodgepole pines at Palmer Creek. Figure 1 shows the diameter of all available and attacked trees in each canopy foliage score category. In score category 6 (the beginning of the time encompassed by this study) the average diameter of all lodgepole pine available as host trees was significantly greater than that of whitebark pine at Mt. Edith and Palmer Creek (Independent Samples T-Test, Mt. Edith: $t_{(272)} = -5.58$, $p < 0.001$; Palmer Creek: $t_{(189)} = -3.80$, $p < 0.001$; Vipond Park: not significant). The average diameter of lodgepole pine at Mt. Edith was 26.1 ± 1.02 cm versus 18.5 ± 2.11 cm for whitebark pine, the average diameter of lodgepole pine at Palmer Creek was 31.1 ± 0.92 cm versus 21.5 ± 1.33 cm in whitebark pine, and the average diameter of lodgepole pine at Vipond Park was 19.85 ± 1.70 cm versus 18.7 ± 1.84 cm in whitebark pine (mean ± standard deviation). Linear regression was used to evaluate change in the diameter of attacked trees over score categories.

For each site, binary logistic regression was used to predict mountain pine beetle attack, using tree species and diameter as explanatory variables (Table 2). We calculated the change in tree diameter that that predicted the same change in probability of beetle attack as a change in tree species, by dividing the regression coefficient for species by the regression coefficient for diameter (Table 2). Separate regressions were performed for each canopy foliage score at each site to evaluate whether these predictors changed over time as the abundance of each tree species changed (Table 3). The first of these separate regressions modeled attack in trees in the oldest canopy foliage score category. Trees with score 6 were “attacked” and trees with scores 0-5 were “un-attacked.” Our next regression modeled attack in trees with score 5; trees with scores 0-4 were un-attacked and trees with score 6 were excluded. Next, trees with score 4 were considered attacked while trees with scores 5 and 6 were excluded, and so on. Our last regression included only the remaining trees - those attacked in 2008 and those that remained un-attacked (scores 1 and 0). In using this technique we assumed that trees with higher scores were always attacked before trees with lower scores at the same site. Statistical analyses were performed in PASW Statistics 18 (IBM Statistics).

Results

Tree diameter was a significant predictor of mountain pine beetle attack at all three sites, and larger trees were more likely to be attacked than smaller trees (Table 2, Figure 1). Overall, the diameter of attacked lodgepole pine trees was significantly greater than the diameter of attacked whitebark pine trees at both Mt. Edith and Palmer Creek (Independent Samples T-test, Mt. Edith: $t_{(216)} = -5.87$, $p < 0.001$; Palmer Creek: $t_{(84)} = -2.30$, $p = 0.024$; Vipond Park: not significant). The diameter of attacked trees also declined significantly over score categories for whitebark pine at all sites, and for lodgepole pine at Mt. Edith and Vipond Park (Figure 1; $r^2 = 0.061 – 0.288$, $p \leq 0.031$). In separate logistic regressions performed for individual score categories, we found that tree diameter was also a consistent predictor of the probability of mountain pine beetle attack over time. Diameter was significant in most score categories at Mt. Edith and Vipond Park, and in two score categories at Palmer Creek (Table 3).

We found that whitebark pine was more likely to be attacked than lodgepole pine at both Mt. Edith and Palmer Creek, even though whitebark pines tended to be smaller than lodgepole pines (Table 2, Figure 2). We calculated that for the mountain pine beetle, a change in species from whitebark pine to lodgepole pine was equivalent to an increase in diameter of 14.7 cm at

Mt. Edith and 10.7 cm at Palmer Creek (Table 2, Figure 3; Mt. Edith: $y = 1 / (1 + e^{-(1.306 + 0.214x_1 - 3.140x_2)})$, Palmer Creek: $y = 1 / (1 + e^{-(-2.099 + 0.148x_1 - 1.582x_2)})$). Although there was no significant difference in the probability of beetle attack between species at Vipond Park, at the other two sites, smaller whitebark pine appeared to be equivalent to larger lodgepole pines as a host tree for the mountain pine beetle. Unfortunately, we did not possess the statistical power to detect the importance of species over time at Vipond Park or Palmer Creek, due to a low number of attacked lodgepole pine or to a low overall number of lodgepole pine at those sites, but whitebark pine was significantly more likely to be attacked than lodgepole pine in most score categories at Mt Edith (Table 3).

Discussion

In agreement with an extensive body of research on mountain pine beetle host selection (e.g. Amman and Cole 1983; Raffa and Berryman 1983; Safranyik 2003), we found that larger diameter trees were more likely to be attacked (Table 2, Figure 1). This pattern was significant across all three sites. We also found that whitebark pine was more likely to be attacked than lodgepole pine at two sites (Table 2, Figure 2), despite the fact that available whitebark pines were of smaller diameter than available lodgepole pines (Figure 1). Further, the mountain pine beetle attacked progressively smaller whitebark pines although larger lodgepole pines were available (Figure 1). Thus, while tree diameter was more often a significant and consistent predictor of mountain pine beetle attack across sites and time (Table 2, Table 3), tree species was also relevant in mountain pine beetle host selection. To our knowledge, this study is the first to evaluate how tree species and diameter influence mountain pine beetle host choice in a mixed stand, and to suggest a role for small diameter whitebark pine trees in maintaining high elevation beetle outbreaks.

An important finding of this study is that mountain pine beetles prefer to attack small diameter whitebark pines even when relatively large lodgepole pines were available nearby (Figure 1). Although beetles typically attack smaller trees when host selection is limited (Robertson et al. 2007), tree diameters greater than 20 cm are considered necessary to sustain mountain pine beetle populations in lodgepole pine stands (Cole and Amman 1969; Amman and Cole 1983). At Mt. Edith, beetle attack was observed in whitebark pines less than 20 cm in diameter in two score categories (Figure 1), and at Mt. Edith and Palmer Creek, we calculated that whitebark pines were as likely to be attacked as lodgepole pines that were 10.7 or 14.7 cm larger in diameter. This suggests that the Mt. Edith and Palmer Creek outbreaks, and perhaps that high elevation mountain pine beetle outbreaks in general, may be prolonged by beetles using host trees that are unexpectedly small.

Beetle selection of smaller whitebark pines is likely due to both the nutritional quality and the defenses of the host tree. When beetles are using lodgepole pine, their selection behavior varies depending on whether the beetles are endemic or eruptive. Eruptive populations are not constrained by tree defenses and are able to select the highest quality host trees regardless of tree vigor or defensive capability (Alcock 1981; Raffa and Berryman 1983; Bentz et al. 1993; Raffa et al. 2005; Raffa et al. 2008; Clark et al 2010; Boone et al. 2011). If whitebark pine provides better nutrition to beetles, by having thicker phloem or higher nutrient concentrations in the phloem, then small whitebark pine may be just as nutritious as larger but better defended lodgepole pines (Six and Adams 2007; Lahr and Sala in prep), leading the mountain pine beetle to attack small diameter whitebark pine even when larger lodgepole pine are still available.

Although the importance of tree diameter and species were consistent across our study sites, future studies would benefit from addressing additional factors that can influence mountain pine beetle host selection. These include differences in tree monoterpenes that may influence beetle attraction and pheromone communication (Wood 1982; Pureswaran et al. 2004; Seybold et al. 2006), and the spatial distribution of tree species (Robertson et al. 2007). At Palmer Creek, in particular, the more recent and moderate beetle pressure and the small number of lodgepole pine may have biased our result that whitebark pine were more likely to be attacked. Overall, however, neither the abundance of whitebark pine nor beetle preference for this species prevented beetle attack of lodgepole pine at any site, thus reinforcing our study results.

Several authors have suggested that mountain pine beetles prefer whitebark pine over lodgepole pine (Baker et al. 1971; Six and Adams 2007). Our study, however, empirically demonstrates beetle preference for whitebark pine where these two species co-occur, and also suggests the importance of small diameter whitebark pine to mountain pine beetle populations in high elevation areas. If mountain pine beetles use tree nutritional quality as a host selection cue, small whitebark pines are equivalent to much larger lodgepole pines as suitable host trees. The presence of smaller but still high quality host trees may enable the mountain pine beetle to persist in stands where outbreaks would otherwise end after all large trees were killed. Over the course of an outbreak, such a difference in host tree species, and the ability to maintain an eruptive population in small diameter host trees, could allow the mountain pine beetle to persist at and disperse from high elevations better than previously believed. In addition to tree defenses, factors specifically related to tree size and species, such as nutritional quality, may therefore be important to consider in understanding mountain pine beetle outbreak progression. This information may improve our understanding of the intensity and duration of mountain pine beetle dynamics in high elevation whitebark pine and potentially in other host species as mountain pine beetle range continues to expand in the future.

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Table 1. Tree canopy foliage scoring system.

Score	Tree Description
0	Un-attacked, green needles
1	Fresh beetle pitch tubes, green needles (attacked in 2008)
2	Canopy color ranges from green-yellow-red needles
3	Canopy primarily bright red, some yellow-green needles
4	Canopy primarily dull red needles
5	Canopy all dull red needles, partial needle loss
6	Considerable or complete needle loss

Table 2. Binary logistic regression showing the probability of mountain pine beetle attack at each site.

Site	**N**	**Predictor**	**B**	**S.E.**	**Wald**	**Sig.**	**Exp(B)**
Edith Peak	274	Constant	1.306	0.595	4.813	0.028	3.692
		Diameter	0.214	0.039	30.586	**0.000**	1.239
		Species	-3.140	0.517	36.870	**0.000**	0.043
Vipond Park	180	Constant	-5.318	0.931	32.626	0.000	0.005
		Diameter	0.267	0.041	42.145	**0.000**	1.306
		Species	-0.363	0.407	0.795	0.373	0.696
Palmer Creek	191	Constant	-2.099	0.742	8.009	0.005	0.123
		Diameter	0.148	0.024	38.327	**0.000**	1.159
		Species	-1.582	0.645	6.008	**0.014**	0.206

Models for each site include tree diameter (cm) and species (whitebark pine, lodgepole pine) as predictors. N = sample size at each site. Model output shows parameter estimates (B), standard error (S.E.), the Wald test statistic, significance of each predictor indicated in bold (Sig.), and the odds ratio change for each parameter (Exp (B)). For diameter, positive parameter estimates indicate that larger trees are more likely to be attacked by the mountain pine beetle, and for species, negative parameter estimates indicate that whitebark pine is more likely to be attacked than lodgepole pine.

Table 3. Binary logistic regression showing the probability of mountain pine beetle attack at each site and in each canopy foliage score category.

Site	Score	N	Predictor	B	S.E.	Wald	Sig.	Exp(B)
Edith Peak	6	274	Constant	-2.477	0.638	15.081	0.000	0.084
			Diameter	0.081	0.018	19.144	**0.000**	1.084
			Species	-0.950	0.392	5.865	**0.015**	0.387
	5	231	Constant	1.094	0.524	4.354	0.037	2.987
			Diameter	0.081	0.023	12.054	**0.001**	1.084
			Species	-2.611	0.424	37.941	**0.000**	0.073
	4	145	Constant	0.151	0.716	0.044	0.833	1.163
			Diameter	0.119	0.037	10.276	**0.001**	1.126
			Species	-2.811	0.618	20.660	**0.000**	0.060
	3	111	Constant	-1.385	1.345	1.061	0.303	0.250
			Diameter	0.005	0.076	0.005	0.943	1.005
			Species	-0.930	1.042	0.796	0.372	0.395
	2	104	Constant	-4.554	1.897	5.763	0.016	0.011
			Diameter	0.152	0.081	3.491	0.062	1.164
			Species	-1.167	1.123	1.080	0.299	0.311
	1	98	Constant	-2.497	0.932	7.183	0.007	0.082
			Diameter	0.213	0.053	16.066	**0.000**	1.237
			Species	-1.560	0.666	5.488	**0.019**	0.210
Site	**Score**	**N**	**Predictor**	**B**	**S.E.**	**Wald**	**Sig.**	**Exp(B)**
Vipond Park	6	180	Constant	-4.581	1.280	12.816	0.000	0.010
			Diameter	0.119	0.035	11.814	**0.001**	1.127
			Species	-0.599	0.636	0.887	0.346	0.549
	5	166	Constant	-6.931	1.286	29.051	0.000	0.001
			Diameter	0.137	0.033	17.666	**0.000**	1.147
			Species	1.236	0.519	5.682	**0.017**	3.443
	4	143	Constant	13.811	5394.679	0.000	0.998	-
			Diameter	0.125	0.041	9.358	**0.002**	1.133
			Species	-18.860	5394.678	0.000	0.997	0.000

	3	134	Constant	-8.162	1.674	23.762	0.000	0.000
			Diameter	0.288	0.066	18.715	**0.000**	1.333
			Species	0.252	0.594	0.179	0.672	1.286
	2	112	Constant	-3.010	1.425	4.462	0.035	0.049
			Diameter	0.109	0.068	2.586	0.108	1.115
			Species	-1.095	0.900	1.479	0.224	0.335
	1	103	Constant	-4.331	1.765	6.018	0.014	0.013
			Diameter	0.086	0.078	1.210	0.271	1.090
			Species	-0.144	0.993	0.021	0.885	0.866

Site	Score	N	Predictor	B	S.E.	Wald	Sig.	Exp(B)
Palmer Creek	6	191	Constant	-5.627	1.187	22.484	0.000	0.004
			Diameter	0.127	0.029	19.751	**0.000**	1.136
			Species	-0.429	0.839	0.261	0.609	0.651
	5	174	Constant	-2.457	0.999	6.053	0.014	0.086
			Diameter	0.097	0.024	16.123	**0.000**	1.102
			Species	-1.515	0.927	2.672	0.102	0.220
	4	145	Constant	15.060	10492.070	0.000	0.999	-
			Diameter	0.067	0.037	3.312	0.069	1.069
			Species	-19.225	10492.070	0.000	0.999	0.000
	3	136	Constant	13.978	10354.880	0.000	0.999	-
			Diameter	0.089	0.042	4.564	**0.033**	1.093
			Species	-19.066	10354.880	0.000	0.999	0.000
	2	130	Constant	12.260	10520.650	0.000	0.999	-
			Diameter	0.066	0.073	0.827	0.363	1.069
			Species	-17.815	10520.650	0.000	0.999	0.000
	1	128	Constant	-3.000	0.945	10.066	0.002	0.050
			Diameter	0.104	0.030	12.433	**0.000**	1.110
			Species	-0.871	0.812	1.151	0.283	0.419

Canopy foliage score categories are described in Table 1. Separate models for each score included tree diameter and species (whitebark pine, lodgepole pine) as predictors. N = sample size. Model output shows parameter estimates (B), standard error (S.E.), the Wald test statistic, significance of each predictor indicated in bold (Sig.), and the parameter log likelihood (Exp (B). For diameter, positive parameter estimates indicate that larger trees are more likely to be attacked by the mountain pine beetle, and for species, negative parameter estimates indicate that whitebark pine is more likely to be attacked than lodgepole pine.

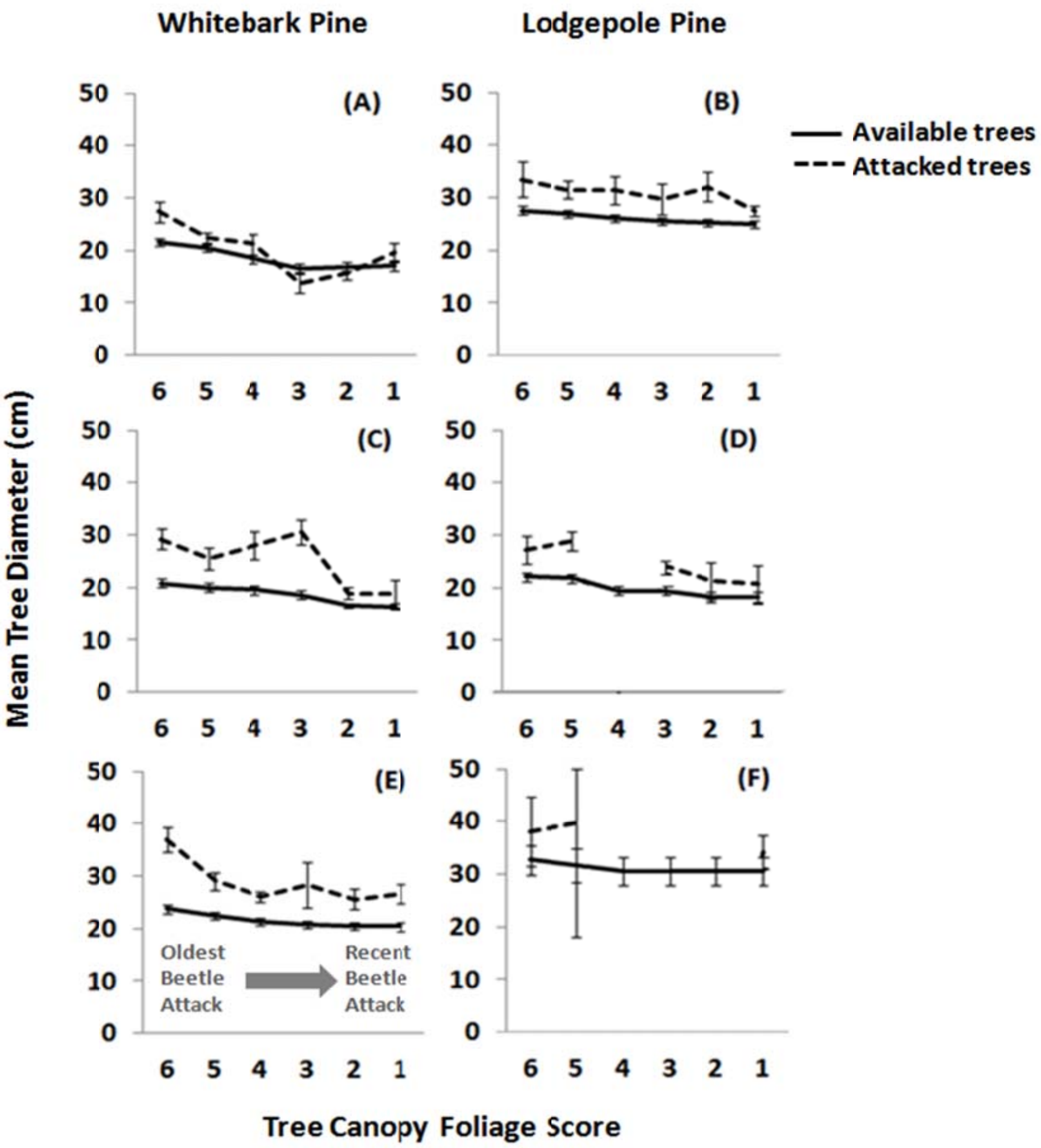

Figure 1. Diameter (mean ± standard error) of trees in each canopy foliage score category at Mt. Edith (A & B), Vipond Park (C & D), and Palmer Creek (E & F). Solid lines indicate the mean diameter available trees and dashed lines indicate the mean diameter of attacked trees in each canopy foliage score category. Lines are discontinuous if beetle attack did not occur in a given score. The progression of time is shown by the arrow in panel B, going from the oldest mountain pine beetle attacks (score = 6) to the most recent (score = 1).

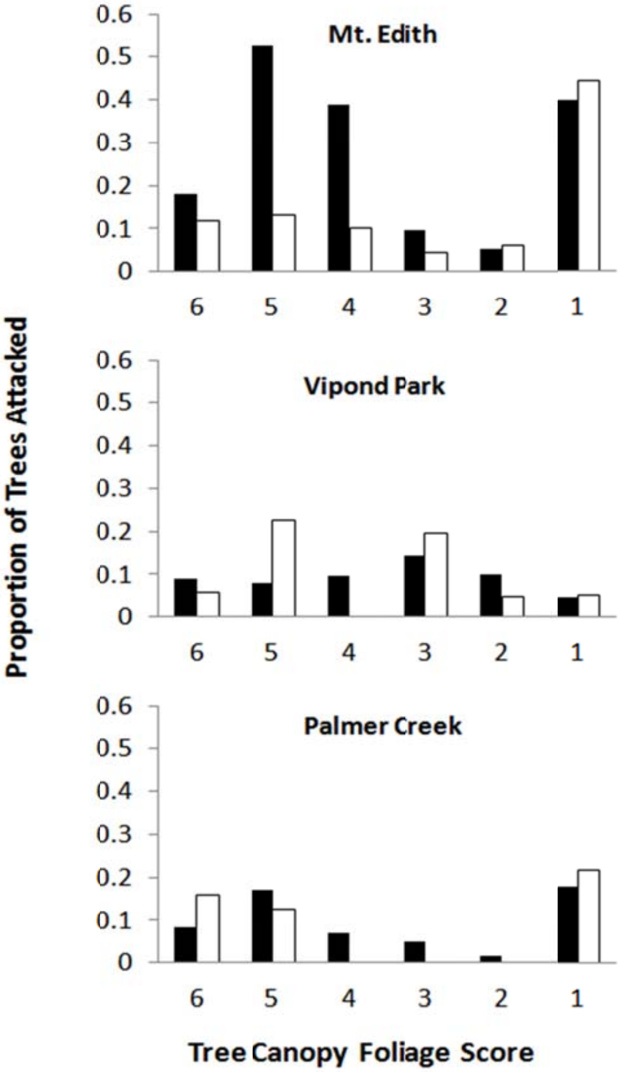

Figure 2. For each species, the proportion of trees of that species attacked by the mountain pine beetle is shown for each canopy foliage score category at Mt. Edith, Vipond Park, and Palmer Creek. Closed bars indicate whitebark pine and open bars indicate lodgepole pine.

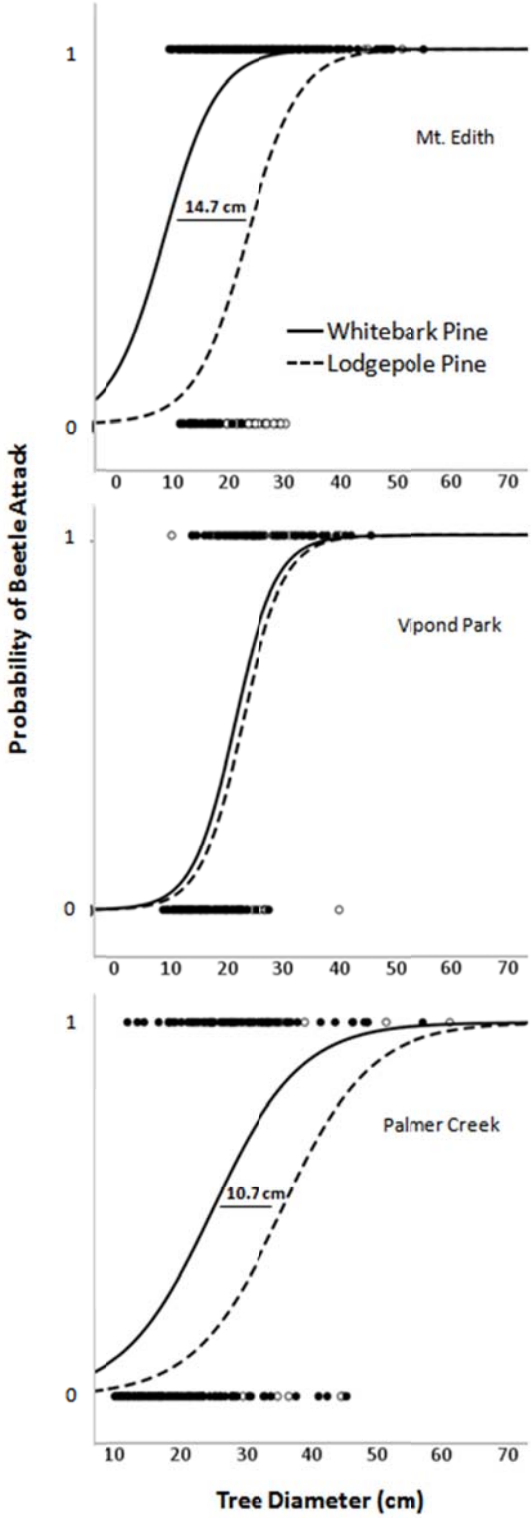

Figure 3. The overall probability of mountain pine beetle attack at Mt. Edith, Vipond Park, and Palmer Creek in relation to tree species and diameter (1 = attacked, 0 = un-attacked). Closed

circles indicate whitebark pine and open circles indicate lodgepole pine. Fit lines represent the logistic regression for each species; solid lines indicate whitebark pine and dashed lines indicate lodgepole pine. At Mt. Edith and Palmer Creek, species was a significant predictor of the probability of beetle attack, and the amount of diameter change equivalent to a change in species is shown between the fit lines (parameter estimates for this calculation are given in Table 2).

CHAPTER 3

ELEVATION MAY AFFECT NUTRITIONAL QUALITY OF HOST TREES FOR MOUNTAIN PINE BEETLES AND FUNGI

Abstract The mountain pine beetle (*Dendroctonus ponderosae*) outbreak in North America is responsible for the loss of over 20 million acres of conifer forests in recent years. Beetles feed upon tree phloem and on beetle-vectored fungi that grow in the phloem, and research has shown that phloem traits and host nutritional quality influence beetle and/or- fungal development. However, factors that affect overall tree nutritional quality have received less attention, despite their potential to influence beetle dynamics in novel environments or less common host species. Here, we investigate whether host tree nutritional quality differs according to elevation, diameter, and tree species. We sampled trees in two diameter classes at two elevations, and measured non-structural carbohydrates, lipids, nitrogen, and phosphorous in the phloem and sapwood of a common host, lodgepole pine (*Pinus contorta*), and an uncommon host but important keystone species in the Rocky Mountains, whitebark pine (*Pinus albicaulis*). For both species, phloem and sapwood stored resource concentrations increased with elevation and tree diameter. Stored resources were generally higher in whitebark pine, where even small diameter trees generally had high resource concentrations relative to lodgepole pine. Phloem and sapwood depth were positively correlated with tree diameter but differed between species; lodgepole pine had more sapwood and whitebark pine had more phloem. Overall, whitebark pine appears to be of higher nutritional quality than lodgepole pine for the mountain pine beetle. This has important management implications for high elevation ecosystems in the Rocky Mountains, where mountain pine beetle access to higher quality host trees may increase beetle outbreaks and threaten whitebark pine.

Keywords *Dendroctonus ponderosae,* mountain pine beetle, *Pinus albicaulis, Pinus contorta,* tree stored resources

Introduction

Trees that grow at high elevations often have greater resource stores than trees found at lower elevations, which may benefit the trees but also benefit the insect herbivores that feed on them. Resource storage can buffer trees against environmental stochasticity (Li et al. 2001), improve reproductive success (McDowell et al. 2000, Miyazaki et al. 2002, Ichie et al. 2005) and augment tree defenses (Franceschi et al. 2005, Guérard et al. 2007), but increased carbon and nutrient storage may also provide a better food source for insect herbivores. Nitrogen and phosphorus, which are important limiting nutrient for insects (Mattson 1980, White 1993, Sterner and Elser 2002, Behmer 2009), may accumulate in high elevation trees due to their slow growth and long intervals between reproductive events (Hoch and Körner 2005, Li et al. 2008). Likewise, cold temperatures limit tree growth more than photosynthesis, which can cause non-structural carbohydrates (sugars and starch) and lipids to increase along elevation gradients as photo-assimilates are acquired faster than they can be used (Hoch et al. 2002, Hoch and Körner 2003, Bansal and Germino 2008, Hoch and Körner 2009).

Such elevation-dependent increases in stored tree resources may influence the population dynamics of forest insects such as the mountain pine beetle (*Dendroctonus ponderosae*), an aggressive pest that is currently experiencing unprecedented population outbreaks and range expansion into high elevation forests across western North America (Logan and Powell 2001, Raffa et al. 2008). A growing number of studies indicate that both tree phloem (Ayres et al. 2001, Bleiker and Six 2007, Cook et al. 2010) and sapwood resources (E. Lahr and A. Sala unpublished data) correlate with the performance of beetles and/or beetle-associated fungi. To assess the susceptibility of high elevation forests to mountain pine beetle outbreaks, it is crucial that we understand the factors that influence host tree nutritional quality.

Historically, high elevation forests have been protected from the mountain pine beetle by the same cold temperatures and harsh environmental conditions that cause increased tree resource storage, but warmer winters in recent years have increased beetle range and overwinter survival *(*Logan and Powell 2001, Carroll and Safranyik 2003, Raffa et al. 2008). Because of this the mountain pine beetle now represents a grave threat to high elevation whitebark pine (*Pinus albicaulis* Engelmann), a keystone species in the northern Rocky Mountains of the United States (Logan and Powell 2001, Raffa et al. 2008). Whitebark pine's longevity *(*Loehle 1996, Larson 2001, Black and Colbert 2008), low investment in foliage (Schoettle and Fahey 1994, A. Sala unpublished data), and long intervals between seed crops (McCaughey and Tomback 2001), coupled with the fact that it is a high elevation specialist, suggest that this species may have greater resource storage than the more common lower elevation host of the mountain pine beetle, lodgepole pine (*Pinus contorta* Douglas ex. Louden), where these two species co-occur.

During lower elevation outbreaks, the mountain pine beetle is known to prefer large lodgepole pines with more phloem (Amman and Cole 1983, Amman and Pasek 1986, Zausen et al. 2005, Boone et al. 2011, but see Thomson and Shrimpton 1985), but although lodgepole pine often exceeds whitebark pine in diameter where the two species co-occur, whitebark pine may have thicker phloem (Amman 1982, Six and Adams 2007, but see Baker et al. 1971). Phloem and sapwood resource concentrations may positively influence bark beetle performance, particularly when nutrients are concentrated in the phloem by symbiotic fungi (Ayres et al. 2001). While sapwood resources are not directly accessible to beetles, they may still be an important component of overall tree nutritional quality because beetle-associated fungi are efficient at concentrating limiting nutrients (Ayres et al. 2001, Bleiker and Six 2007, Cook et al. 2010) and are able to grow deep into the sapwood of the tree (Solheim 1995, Solheim and Krokene 1998, E. Lahr and A. Sala unpublished data).

In the context of these recent mountain pine beetle outbreaks and expansion to high elevation forests, it is important to better understand the influence of elevation, diameter, and species on tree nutritional quality. We measure the concentrations of non-structural carbohydrates (NSC), lipids, nitrogen, and phosphorus in tree phloem and sapwood, as well as the depth of phloem and sapwood tissue, in trees at two elevations in the Rocky Mountains of Idaho, U.S.A. At each elevation we compare whitebark pine and lodgepole pine in small and large diameter classes. We focus on the following three questions: 1) Do stored resources increase with elevation for both tree species? 2) Do larger trees have greater phloem and sapwood stored resources than smaller trees? 3) Do whitebark pines, which are able to grow at higher elevations, have greater phloem and sapwood stored resources than lodgepole pines where the two species co-occur?

Methods

Study system

Whitebark pine *(Pinus albicaulis)* is a keystone species that occurs in the northern Rocky, Cascade, and Sierra Nevada Mountains from approximately 2000-3500 m elevation. Lodgepole pine (*Pinus contorta*) occurs throughout the western United States and Canada from approximately 1000-3000 m elevation and frequently overlaps with whitebark pine at its upper range limits. Lodgepole pine is a common host of the mountain pine beetle (Coulson and Witter 1984). The mountain pine beetle (*Dendroctonus ponderosae)* is a highly aggressive bark beetle (Coulson and Witter 1984, Paine et al. 1997, Raffa et al. 2008), and recent outbreaks have been attributed to warmer temperatures that have improved overwinter survival and allowed the beetle to expand its range northwards and upwards in elevation (Logan and Powell 2001, Carroll and Safranyik 2003). Adult beetles carry fungal spores that are deposited in the tree during egg-laying (Whitney and Farris 1970), and beetle larvae feed on phloem tissue and fungal hyphae while new adults feed on fungal spores (Six and Paine 1998, Adams and Six 2007). Fungi feed on phloem and sapwood stored carbon (Barras and Hodges 1969, Lahr and Sala in prep), and benefit beetles by concentrating nitrogen and phosphorus and by providing sterols (Ayres et al. 2001, Bentz and Six 2006, Bleiker and Six 2008, Cook et al. 2010). The fungal species most closely associated with the mountain pine beetle are *Grosmannia clavigera* and *Ophiostoma montium* (Six and Paine 1998, Six and Wingfield 2011).

Study site

Sample collection occurred in September 2009, in the Lemhi Mountains, Idaho, U.S.A. (44°26’01.19” N, 113°19’12.38” W). Mean daily average air temperatures at the study site ranged from -20.2 °C – 5.9 °C in January and from 5.6 °C – 17.4 °C in July. Total precipitation in 2009 prior to sampling was 889 mm. Samples were collected at 2400 m and 2900 m elevation on the same mountain slope. These elevations were selected because they represented the boundaries of where whitebark pine and lodgepole pine continuously co-occurred. At 2400 m, the forest contained closely spaced whitebark pine, lodgepole pine, and sub-alpine fir, and little understory or pine regeneration occurred. At 2900 m, the forest contained evenly spaced whitebark pine and lodgepole pine with an open canopy and no understory. Abiotic factors such as temperature and precipitation usually vary with elevation, but in this study we were interested in the potential for elevation and its correlated abiotic factors to influence tree quality in the context of mountain pine beetle range expansion. At each site diameter at breast height (DBH; measured 1.4 m above the ground) was recorded and 7-10 trees of each species were sampled in each of two diameter classes: small (< 18 cm DBH) and large (> 21 cm DBH). These diameter thresholds were selected because tree diameters greater than 20 cm are considered necessary to sustain mountain pine beetle populations in lodgepole pine stands (Cole and Amman 1969, Amman and Cole 1983). Small trees had a diameter range of 10.3 – 18.0 cm, and large trees had a diameter range of 21.0 – 46.9 cm. Sapwood depth, a linear measurement of the amount of water conducting xylem, was also measured at this time. In July 2011, additional measurements of diameter and phloem depth were taken for 10 trees per species, elevation and diameter class.

Sample collection and biochemical analysis

In 2009, from 1-3 sapwood cores per tree were obtained at breast height with a 5 mm increment borer, and one phloem sample per tree was obtained using a 30 mm arch punch. Sapwood depth was measured using a ruler, and phloem depth was measured using digital calipers. Mean sapwood and phloem depth are shown in Table 1. Sapwood cores for each tree were pooled, and samples were transported to the lab on ice and then oven dried at 75 °C for 48 hours. Phloem samples were ground to powder in a blade coffee grinder, and sapwood samples were ground to powder using a Wylie Mill with a size 40 screen (Thomas Scientific, Swedesboro, New Jersey, U.S.A.) followed by a Genogrinder 2000 (OPS Diagnostics LLC, Lebanon, New Jersey, U.S.A.).

Phloem and sapwood non-structural carbohydrates (glucose, fructose, sucrose, and starch), nitrogen, and phosphorus, and sapwood lipids (acylglycerols) were measured. Non-structural carbohydrates (NSC) were analyzed using the photometric method of Hoch et al. (2002). Briefly, 12-14 mg of wood powder was extracted in 1.6 mL distilled water at 100 °C for one hour. An aliquot of this water was used to determine low molecular weight carbohydrates following enzymatic breakdown of fructose and sucrose to glucose. Following enzymatic breakdown of starch to glucose by a fungal amylase (‘Clarase,’ Genencor International Inc., Rochester, New York, U.S.A.) at 40 °C overnight, and the conversion of glucose to gluconate-6-phosphate, the total glucose concentration was determined in a 96-well microplate reader at 340 nm (model EL800, BioTek Instruments Inc, Winooski, Vermont, U.S.A). Sapwood lipids were analyzed using a similar photometric analysis according to Hoch et al. (1999). Briefly, 10-14 mg of wood powder was extracted in 1 mL aqueous NaOH for 30 minutes and glycerol was converted to glycerol-3-phosphate. The amount of liberated glycerol was determined in a 96-well microplate reader at 340 nm (model EL800, BioTek Instruments Inc, Winooski, Vermont, U.S.A). Nitrogen was measured by the staff of the University of California Davis Stable Isotope Facility using an elemental analyzer and mass spectrometer, following sample combustion. Phosphorus was measured by the staff of the Colorado State University Soil Water and Plant

Testing Laboratory using inductively coupled plasma-atomic emission spectroscopy, following acid digest of the sample.

Data analysis

Univariate analysis of variance was used to assess the effects of elevation, tree species, and tree diameter on stored resource concentrations in the phloem and sapwood (Table 2, Figure 1). Analyses were performed on resource concentrations, rather than the total amount of resources, to reflect the nutritional quality of the immediate environment experienced by beetles and fungi (e.g. Ayres et al 2001, Bleiker and Six 2007). Explanatory variables included elevation (2400 m, 2900 m), tree species (whitebark pine, lodgepole pine), tree diameter class (small, large), and interaction terms. Sapwood depth (Table 1) was included as a covariate. Phloem depth was not measured in 2009 and is not included as a covariate in this analysis. Response variables included the concentration of phloem and sapwood NSC, nitrogen, and phosphorus, and sapwood lipids. Univariate analysis of variance was also used to assess the effects of elevation, tree species, and tree diameter class on sapwood depth in 2009 and phloem depth in 2011 (Table 3, Figure 2). Statistical analyses were done with PASW Statistics 18 (IBM Statistics).

Results

Elevation, tree species, and tree diameter all contributed to differences in overall tree nutritional quality (Table 2, Figure 1). Phloem and sapwood resource concentrations were generally higher at 2900 m relative to 2400 m (phloem NSC: $F_{(1, 55)} = 18.956$, $p = 0.000$, phloem phosphorus: $F_{(1,}$

$_{55)}$ = 22.657, p = 0.000; sapwood lipids: $F_{(1, 63)}$ = 16.213, p = 0.000; sapwood NSC: $F_{(1, 63)}$ = 53.804, p = 0.000; sapwood phosphorus: $F_{(1, 57)}$ = 12.861, p = 0.001) and resource concentrations were generally higher in larger trees (phloem nitrogen $F_{(1, 55)}$ = 10.540, p = 0.002; sapwood NSC: $F_{(1, 63)}$ = 17.373, p = 0.000; sapwood nitrogen: $F_{(1, 63)}$ = 10.451, p = 0.002). Stored resources were also generally higher in whitebark pine (phloem nitrogen: $F_{(1, 55)}$ = 11.485, p = 0.001; phloem phosphorus: $F_{(1, 55)}$ = 4.069, p = 0.049; sapwood lipids: $F_{(1, 63)}$ = 4.590, p = 0.036; sapwood NSC: $F_{(1, 63)}$ = 10.367, p = 0.002; sapwood nitrogen: $F_{(1, 63)}$ = 60.993, p = 0.000). Except for nitrogen, which was not influenced by elevation, elevation and tree diameter positively influenced resource concentrations, which were generally also higher in whitebark pine (Table 2, Figure 1). The influence of species on sapwood resources was more consistent than the influence of species on phloem resources, as interactions between species and diameter and between species and elevation obscured the overall effect of species on phloem NSC (Table 2). Small whitebark pine also had resource concentrations greater than or equivalent to large lodgepole pine for sapwood lipids and NSC, sapwood and phloem nitrogen, and phloem phosphorus (Figure 1).

Phloem and sapwood depth were influenced by tree species and diameter, but not elevation (Table 3, Figure 2). Larger trees had greater phloem depth ($F_{(1, 73)}$ = 4.023, p = 0.049), and whitebark pine had more phloem than lodgepole pine ($F_{(1, 73)}$ = 7.626, p = 0.007). Small whitebark pines were equivalent to large lodgepole pines in phloem depth (Figure 2). Larger trees also had greater sapwood depth ($F_{(1, 63)}$ = 38.360, p = 0.000), and lodgepole pine had more sapwood than whitebark pine ($F_{(1, 63)}$ = 17.201, p = 0.000).

Discussion

We found that resource storage, which is thought to be an important determinant of host quality for insect herbivores (Mattson 1980, Awmack and Leather 2002, Behmer 2009), increased with both elevation and tree diameter, and that whitebark pines had higher resource concentrations than lodgepole pines (Table 2, Figure 1). If stored resources influence host nutritional quality, and therefore mountain pine beetle performance, these data suggest that large, high elevation whitebark pines may be a superior host for the mountain pine beetle because of their relatively greater concentrations of stored carbon compounds and mineral nutrients (Figure 1). Beetles preferentially select trees with larger diameters (Amman and Cole 1983, Safranyik 2003), and for lodgepole pines, diameters greater than 20 cm are considered necessary to sustain mountain pine beetle populations (Amman and Cole 1983). However, we have observed that mountain pine beetles will attack whitebark pines less than 20 cm in diameter even when larger lodgepole pines are available (E. Lahr and A. Sala unpublished data). An important finding in this study is that small diameter whitebark pines (diameter < 18 cm) have relatively high concentrations of stored resources (Figure 1), which again indicates their suitability as host trees for the mountain pine beetle. Furthermore, the phloem depth of small whitebark pines was equal to or greater than that of large lodgepole pines (Figure 2). Because beetles prefer trees with thicker phloem tissue (Amman 1982, Six and Adams 2007), these results suggest that both small and large whitebark pines may be valuable high elevation hosts (Figure 1, Figure 2). Our results have important management implications, since the nutritional quality of high elevation whitebark pine could influence the rate, magnitude, and persistence of high elevation mountain pine beetle outbreaks in unexpected ways.

Most previous bark beetle-fungal studies have focused solely on phloem resource concentrations (e.g. Ayres et al. 2001, Bleiker and Six 2007, Cook et al. 2010), but sapwood resources may also be an important component of tree nutritional quality. Although mountain pine beetles develop in the phloem, beetle-associated fungi grow deep into tree sapwood and can utilize sapwood resources (Solheim 1995, Solheim and Krokene 1998, E. Lahr and A. Sala unpublished data). Sapwood lipids, non-structural carbohydrates, and phosphorus were higher at 2900 m relative to 2400 m elevation, and although lodgepole pines had greater sapwood depth (Table 3), whitebark pines had higher overall resource concentrations (Table 2, Figure 1). Thus, for fungi growing in the sapwood, uptake of limiting resources may be significantly more efficient in high elevation whitebark pine than in lodgepole pine.

Nitrogen is one of the most frequently limiting resources in insect development (Mattson 1980, White 1993, Behmer 2009). Some studies have shown that tree stored nitrogen increases with elevation, perhaps due to lower investment in foliage or long intervals between reproductive events (Hoch and Körner 2005, Li et al. 2008), while other studies suggest that stored nitrogen decreases with elevation, perhaps due to limited microbial activity in the soil and nutrient availability for trees (Richardson 2004). Our data do not indicate that elevation influences nitrogen concentrations in whitebark pine or lodgepole pine (Table 2, Figure 1). However, nitrogen concentrations differed between tree species, again suggesting that whitebark pines provide better nutrition to beetles than do lodgepole pines (Table 2, Figure 1).

Our data further suggest that stored phosphorus and carbon compounds are the primary contributors to altitudinal differences in tree nutritional quality, particularly in whitebark pine (Table 2, Figure 1). The role of phosphorus in bark beetle performance has received much less attention than the role of nitrogen (Hodges and Lorio 1969, Bleiker and Six 2007, Cook et al.

2010, but see Ayres et al. 2001), but phosphorus availability is thought to play an important part in insect development and growth (Sterner and Elser 2002), and higher phloem phosphorus concentrations in areas colonized by fungi have been correlated with improved beetle performance (Ayres et al 2001). Likewise, the role of stored carbon has received less attention, and even though NSC and lipids are not limiting in plants, higher concentrations may provide a better food source by lessening the amount of structural tissue that beetles consume.

In addition to their influence on nutritional quality, stored resources can also help trees defend themselves against bark beetles. Trees defend themselves by maintaining adequate stores of resin, which they use to expel beetles, and by forming necrotic lesions in the phloem to contain fungi (Raffa and Berryman 1983, Krokene et al. 1999, Franceschi et al. 2005). These defenses are thought to rely on the remobilization of stored carbon and nitrogen from the phloem and sapwood (Christiansen and Ericsson 1986, Dunn and Lorio 1991, Guérard et al. 2007). While high elevation plants tend to experience less herbivore pressure and have decreased chemical defenses (Salmore and Hunter 2001), there is surprisingly little information regarding whether elevation influences the overall ability of a tree to allocate resources to defense. The exact recipe for successful tree defense against bark beetles is also unclear; for example, differences in carbohydrate levels are not consistently correlated with tree susceptibility to beetle attack, tree resin production capacity, or the size of necrotic lesions formed in response to fungal colonization (Miller and Berryman 1986, Christiansen and Ericsson 1986, Dunn and Lorio 1991). Lodgepole pine can successfully defend itself against mountain pine beetle attack when beetle population densities are low (Raffa and Berryman 1983), but high elevation whitebark pine was historically inaccessible to the mountain pine beetle and is therefore thought to be less defended than lodgepole pine and subsequently more susceptible to beetle attack (Perkins and

Roberts 2003, Raffa et al. 2008). Whitebark pine is consequently at risk in two ways; first because of its potential inability to reallocate resources to defense, and second because tree nutritional quality remains high without defense as a sink for stored resources.

Our study highlights the importance of understanding how stored resources in trees may affect the mountain pine beetle and its symbiotic fungi. Our data suggest that elevation and tree nutritional quality are positively correlated, and because of this, high elevation whitebark pine may be particularly susceptible to mountain pine beetle attack. Moreover, whitebark pines of any diameter appear to be at least as nutritious as much larger lodgepole pines. Insect survival at elevation or latitudinal range limits is often restricted by energy requirements (Pullin 1987, Ohtsu et al. 1995, Bentz et al. 2001), and improved tree nutritional quality could therefore have important effects on individual beetle survival and on the progress of high elevation mountain pine beetle outbreaks. The interpretation of our results hinges upon the relationship between stored resources and both beetle and fungal performance, as suggested by several recent studies (Ayres et al. 2001, Bleiker and Six 2007, Cook et al. 2010, E. Lahr and A. Sala unpublished data). However, further research on the ability of whitebark pine to reallocate stored resources to defense, on the value of stored phosphorus and carbon compounds to beetle or fungal development and seasonal variation in these resources, and on beetle and fungal performance along an elevation gradient would be a valuable addition to our current understanding of the importance of this species to the mountain pine beetle. In conclusion, our observations of increased resource storage with elevation, and of the high nutritional quality of whitebark pine, may have important implications for understanding the rate, magnitude, and persistence of high elevation mountain pine beetle outbreaks, and for the future successful management of whitebark pine in the Rocky Mountains.

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Table 1. Sapwood and phloem depth (mean ± standard deviation) for whitebark pine and lodgepole pine.

Elevation	Species [a]	Sapwood (mm)	Phloem (mm)
2400 m	Whitebark Pine		
	Small	17 ± 7	2.39 ± 0.8
	Large	32 ± 8	2.84 ± 0.9
	Lodgepole Pine		
	Small	28 ± 6	2.18 ± 0.6
	Large	52 ± 18	2.41 ± 0.7
2900 m	Whitebark Pine		
	Small	26 ± 7	2.69 ± 0.6
	Large	33 ± 7	2.82 ± 0.6
	Lodgepole Pine		
	Small	26 ± 13	2.08 ± 0.6
	Large	46 ± 17	2.49 ± 0.5

[a] For each tree species, small diameter trees are < 18 cm, and large diameter trees are > 21 cm.

Table 2. ANOVA for the effects of elevation, tree species, and tree diameter class on sapwood and phloem stored resources.

		Sapwood		Phloem	
		F	p	F	p
Lipids	Sapwood Depth (covariate)	0.104	0.748	-	-
	Elevation	16.213	**0.000**	-	-
	Species	4.590	**0.036**	-	-
	Diameter	2.872	0.095	-	-
	Elevation × Species	4.505	**0.038**	-	-
	Elevation × Diameter	0.020	0.887	-	-
	Species × Diameter	0.331	0.567	-	-
	Elevation × Species × Diameter	4.428	**0.039**	-	-
Non-structural Carbohydrates	Sapwood Depth (covariate)	2.411	0.126	0.218	0.643
	Elevation	53.804	**0.000**	18.956	**0.000**
	Species	10.367	**0.002**	3.808	0.056
	Diameter	17.373	**0.000**	0.000	0.987
	Elevation × Species	0.137	0.713	13.995	**0.000**
	Elevation × Diameter	0.004	0.949	0.016	0.899
	Species × Diameter	1.447	0.234	5.905	**0.018**
	Elevation × Species × Diameter	1.828	0.181	1.106	0.298
Nitrogen	Sapwood Depth (covariate)	5.457	**0.023**	2.405	0.127
	Elevation	0.016	0.899	0.092	0.763
	Species	60.993	**0.000**	11.485	**0.001**
	Diameter	10.451	**0.002**	10.540	**0.002**
	Elevation × Species	3.438	0.068	3.520	0.066
	Elevation × Diameter	3.903	0.053	2.028	0.160
	Species × Diameter	4.093	**0.047**	0.347	0.558
	Elevation × Species × Diameter	1.495	0.226	0.609	0.439
Phosphorus	Sapwood Depth (covariate)	2.311	0.134	5.776	**0.020**
	Elevation	12.861	**0.001**	22.657	**0.000**
	Species	0.156	0.694	4.069	**0.049**
	Diameter	0.026	0.873	2.695	0.106
	Elevation × Species	0.277	0.601	13.125	**0.001**
	Elevation × Diameter	0.026	0.871	2.463	0.122
	Species × Diameter	9.945	**0.003**	0.305	0.583
	Elevation × Species × Diameter	4.217	**0.045**	0.007	0.933

ANOVA factors include: elevation (2400 m, 2900 m), tree species (whitebark pine, lodgepole pine), tree diameter class (small < 18 cm, large > 21 cm), and all interactions. Sapwood depth is a covariate. Bold values indicate significance ($p < 0.05$).

Table 3. ANOVA for the effects of elevation, tree species, and tree diameter class on sapwood and phloem depth (mm).

	Sapwood Depth		Phloem Depth	
	F	p	F	p
Elevation	0.060	0.808	0.310	0.580
Species	17.201	**0.000**	7.626	**0.007**
Diameter	38.360	**0.000**	4.023	**0.049**
Elevation × Species	2.631	0.110	0.429	0.515
Elevation × Diameter	1.046	0.310	0.079	0.779
Species × Diameter	4.205	**0.044**	0.092	0.763
Elevation × Species × Diameter	0.084	0.772	1.117	0.294

ANOVA factors include: elevation (2400 m, 2900 m), tree species (whitebark pine, lodgepole pine), tree diameter class (small < 18 cm, large > 21 cm), and all interactions. Bold values indicate significance ($p < 0.05$).

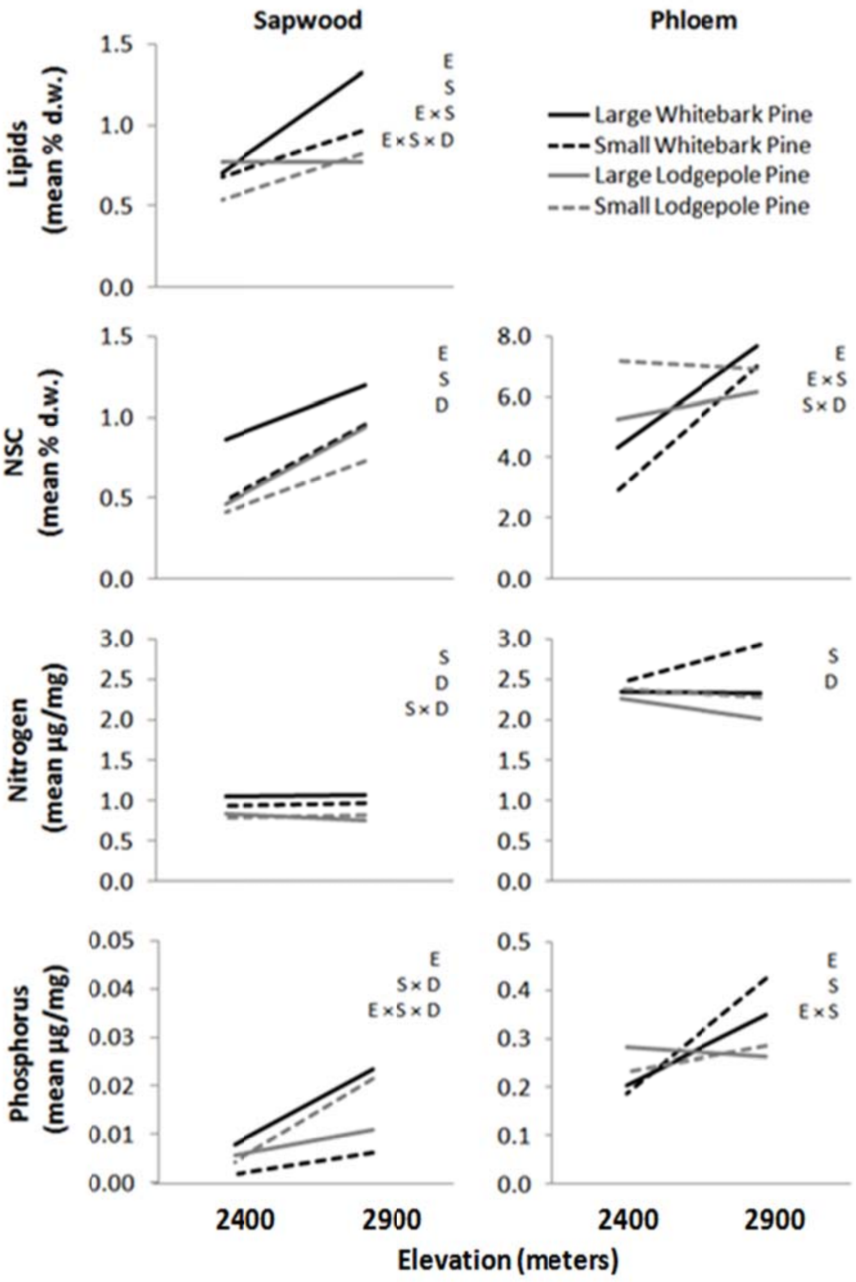

Figure 1. Stored resources in the sapwood and phloem of whitebark pine and lodgepole pine from two diameter classes (small, large) at two elevations (2400 m, 2900 m). Letters indicate ANOVA significance (Table 2). ANOVA factors include elevation (E), tree species (S), diameter class (D), interaction terms, and sapwood depth as a covariate (not shown). Note the different y-axes for sapwood and phloem non-structural carbohydrates (NSC) and phosphorus.

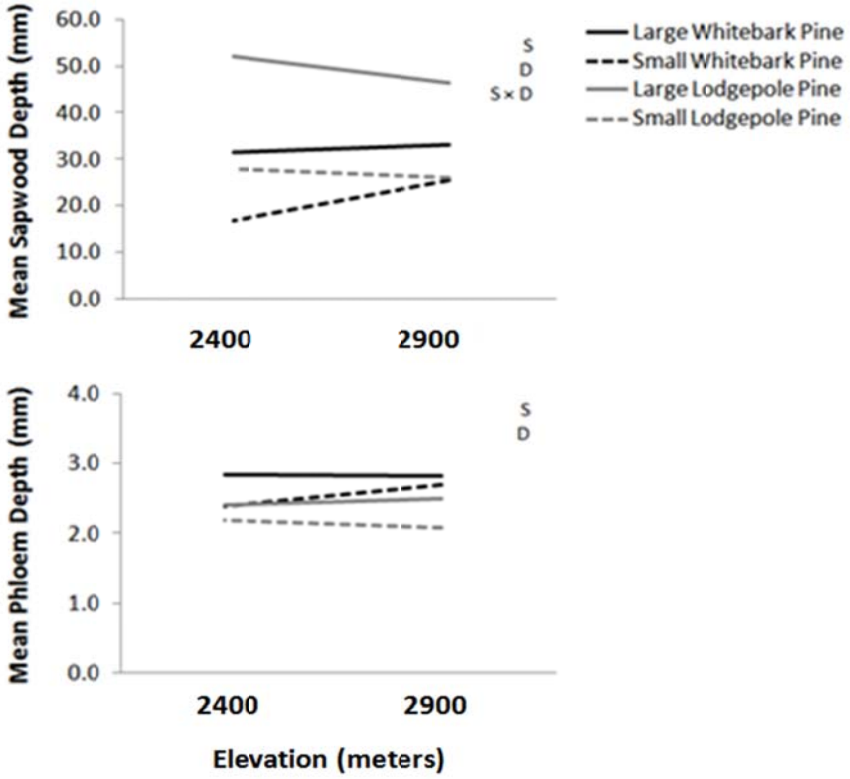

Figure 2. Mean sapwood and phloem depth in whitebark pine and lodgepole pine from two diameter classes (small, large) at two elevations (2400 m, 2900 m). Letters indicate ANOVA significance (Table 3). ANOVA factors include elevation (E), tree species (S), diameter class (D), and interaction terms.

CHAPTER 4

RESOURCE DYNAMICS INFLUENCE TREE SUSCEPTIBILITY TO A FUNGUS ASSOCIATED WITH THE BARK BEETLE *IPS TYPOGRAPHUS*

Abstract Bark beetles and their associated fungi are among the greatest natural threats to conifers worldwide. Conifers have potent defenses, but resistance to fungal pathogens may be complicated by the potential for tree stored resources to enhance fungal performance as well as tree defense. Here, we develop a conceptual model of the relationship between tree resource dynamics and susceptibility to a fungal pathogen. We then evaluate the effects of *Ceratocystis polonica*, a phytopathogenic fungus vectored by the spruce bark beetle *Ips typographus,* on phloem and sapwood nitrogen, non-structural carbohydrates (NSC), and lipids before and after trees were attacked by *I. typographus* or inoculated with *C. polonica.* We evaluate tree susceptibility by measuring phloem lesions and percentage of necrotic phloem occurring following treatments. We found that phloem NSC and sapwood lipids declined in fungal inoculated trees relative to beetle attacked and control treatments, and that while initial resource concentrations were unrelated to tree susceptibility to *C. polonica*, significant negative correlations occurred between tree susceptibility and declines in nitrogen, NSC, and lipids over time. Consistent with our conceptual model, the larger resource declines observed in more susceptible, less resistant trees (i.e. those with larger lesions or a larger percentage of necrotic phloem), suggest that resource depletion may be caused by fungal consumption rather than by tree resistance. Our data do not suggest that *C. polonica* redistributes tree resources to provide nutritional benefits to *I. typographus*, although resource uptake by the fungus could indirectly benefit beetles if it precludes tree resource allocation to resistance. A better understanding of

how tree resource dynamics influence susceptibility to bark beetle-associated fungi could improve our understanding of tree defense during bark beetle outbreaks.

Introduction

Symbiotic relationships between blue-stain fungi and bark beetles have fascinated researchers since they were first observed more than 100 years ago (VonSchrenk 1903; Craighead 1928; Paine et al. 1997; Klepzig and Six 2004; Six and Wingfield 2011), and conifer susceptibility to and resistance against bark beetles and fungi has been the subject of much research (e.g. Berryman 1972; Raffa and Berryman 1982, 1983; Dunn and Lorio 1991; Krokene et al. 1999; Erbilgin et al. 2006; Scott et al. 2008). While a great deal is known about environmental factors that influence fungal colonization of the host tree, such as temperature, oxygen level, and tree water potential (Solheim and Krokene 1998; Klepzig et al. 2004; Hofstetter et al. 2007; Six and Bentz 2007), less is known about how host tree resource dynamics influence susceptibility to and resistance against fungal pathogens.

Fungal phytopathogenicity may help bark beetles to overcome tree defenses and is one basis for beetle-fungal mutualisms (reviewed by Krokene et al. *in press*). Alternatively, mutualistic fungi may provide important nutritional benefits to their beetle partner instead of engaging tree defenses (Barras 1973; Ayres et al. 2001; Six 2003; Bentz and Six 2006; Bleiker and Six 2007, Six and Wingfield 2011). These different perspectives on the nature of beetle-fungal mutualisms may be complementary rather than mutually exclusive, since ultimately tree defenses must be overcome and resources must be utilized for beetles and fungi to thrive in the host tree. However, tree resistance to fungal pathogens may be complicated by the potential for tree stored resources to enhance fungal performance as well as tree resistance. For example, inducible defenses in the tree and long-term resistance to fungi introduced during beetle attack may be precluded by fungal consumption of tree resources or by fungal redistribution of stored resources to benefit beetles. A better understanding of how resource dynamics influence fungal resource utilization versus tree susceptibility to and resistance against fungal pathogens is necessary to fully understand bark beetle-fungal interactions and their consequences for tree defense during bark beetle outbreaks.

Here, we evaluate the relationship between tree stored resource dynamics and susceptibility to the virulent fungal pathogen *Ceratocystis polonica,* vectored by the spruce bark beetle *Ips typographus*. *Ips typographus* is one of the most aggressive and destructive forest insect pests in Northern Europe and has killed millions of trees in periodic outbreaks (Christiansen and Bakke 1988; Bakke 1989; Wermelinger 2004). Its main host tree, Norway spruce (*Picea abies*), is ecologically and economically important across Europe, and the pathogenicity of *C. polonica* to Norway spruce is well documented (e.g. Christiansen 1985; Krokene et al. 1999; Nagy et al. 2000; Krokene et al. 2003; Erbilgin et al. 2006; Zhao et al. 2010). In southern Norway, where this study occurred, *I. typographus* and *C. polonica* are closely associated (Solheim 1991, Krokene and Solheim 1996), although *I. typographus* is associated with other fungi in different parts of its range (Viiri 1997, Viiri and Lieutier 2004). In this study, we measure stored resources (nitrogen, non-structural carbohydrates (NSC), and lipids) in trees attacked by *I. typographus* or inoculated with *C. polonica*. Control, beetle attack, and fungal inoculation treatments were applied to genetically identical Norway spruce ramets, allowing us to compare resource concentrations and tree susceptibility without the added variability of genetic differences. We were specifically interested (i) in whether tree susceptibility to *C. polonica* correlates with initial resource concentrations or resource change over time; and (ii) in whether changes in phloem and sapwood resources following treatment indicate that *C. polonica* redistributes resources from the sapwood to the phloem.

We developed a conceptual model to help us interpret the relationship between tree resource concentrations and susceptibility to *C. polonica* (Figure 1), that explores three possible scenarios that could occur following fungal inoculation. (1): If stored resources benefit the host tree defensively more than they benefit *C. polonica* as a food source, trees with more initial resources and resource depletion over time may be less susceptible and more resistant to fungal colonization (Figure 1A). (2): If stored resources do not have a net influence on the host tree, tree susceptibility may be independent of initial resource concentrations. Resource depletion may then be the result of complex interactions between trees and fungi. For example, greater resource depletion may occur because trees invest in more successful defenses, or alternatively, susceptible trees may have greater resource depletion because fungi consume resources. (3): If stored resources benefit *C. polonica* more than the host tree, trees with higher initial resource concentrations and resource depletion over time may be more susceptible and less resistant to fungal colonization (Figure 1C). Our conceptual model specifically explores the relationship between host tree resources and *C. polonica*, but the combined effect of both beetles and fungi on resource dynamics (in trees attacked by *I. typographus* vectoring *C. polonica*) may be different that than the effect of only fungi (in trees inoculated with *C. polonica*). In particular, an increase in resource concentrations in tree phloem, where the beetles develop, could indicate that *C. polonica* redistributes tree stored resources in a way that provides nutritional benefits to *I. typographus* (e.g. Ayres et al. 2001; Bleiker and Six 2007). By examining the relationships between initial resource concentrations, resource depletion, and tree susceptibility we may be able to infer whether there is a net benefit of stored resources for trees versus fungi.

Methods

Study site and treatments

Three ramets each from nine Norway spruce clones were selected at Hogsmark Experimental Farm, operated by the Norwegian Forest and Landscape Institute, Ås, Norway, for the following treatments: control, attack by *Ips typographus*, or inoculation with *Ceratocystis polonica* (Table 1). Trees were 51 years old at the time of the experiment. Mean tree diameter at breast height was 20.6 cm and did not differ significantly between treatments. Mean daily temperatures at the study site ranged from 10.4 - 17.4 °C in June, 5.8 - 13.2 °C in August, and 0 - 6.2 °C in November. *Ips typographus* is univoltine; reproduction and larval development occur during the summer months, and the next generation of adult beetles emerges from the host tree in mid-August. New adult beetles hibernate in the ground over the winter, and re-emerge the following spring when maximum daytime temperatures in the spring reach 19-20 °C.

The nine trees in the "attack" treatment were baited with attractant pheromone on May 30, 2010 (Ipslure, Borregaard Inc., Sarpsborg, Norway), and were attacked by *I. typographus* the following week. The nine trees in the "inoculation" treatment were inoculated with *C. polonica* (isolate NFLI 93-208/115) on June 9-10, 2010. Trees were mass-inoculated at a density of 400 inoculations m^{-2} around the main bole, from 0.8 – 2.0 m above the ground. Inoculations were performed by removing a bark plug with a 5 mm cork borer, inserting inoculum, and replacing the bark plug. Inoculum consisted of actively growing mycelium of *C. polonica* cultured on malt agar (2% malt and 1.5% agar). This inoculation density simulates the intensity of fungal inoculation during natural *I. typographus* attack (Christiansen 1985; Krokene and Solheim 1998). The number of fungal inoculations made per tree is shown in Table 1.

The susceptibility of inoculated trees was evaluated by measuring the length of five phloem lesions at the top and at the bottom of the inoculation band on November 1, 2010 (Table 1). Smaller phloem lesions indicate lower tree susceptibility and higher tree resistance to fungal pathogens (e.g. Krokene and Solheim 1998; Christiansen et al. 1999; Krokene et al. 2001). In attacked trees, phloem lesion lengths were too small or inconsistently occurring for measurement, so to obtain comparable data on tree susceptibility across treatments, we measured the percentage of necrotic phloem in a 10 cm band around the circumference of all 18 attacked and inoculated trees on June 9, 2011 (Table 1). Measurements were done outside the zone of stem sampling, i.e. at 1.5 m above the ground in inoculated trees and at 1.75 m above the ground in attacked trees.

Sample collection and biochemical analysis

Phloem and sapwood samples were collected from all trees at three time points; (1) on May 30, 2010, prior to any treatment; (2) on August 18, 2010, shortly before emergence of the next *I. typographus* generation; and (3) on October 16, 2010, near the end of the growing season. At each time point we collected one phloem sample, using a 30 mm diameter arch punch, and 2-3 sapwood samples, using a 5 mm hand increment borer, from each tree at around breast height (1.4 m above the ground). Samples were oven dried at 75 °C for 48 hours, and ground to powder in an IKA A11B grinder followed by an IKA MF10 grinder with a 0.5 mm mesh screen (IKA, Staufen, Germany).

We measured sapwood lipids (acylglycerols) and phloem and sapwood nitrogen and non-structural carbohydrates (NSC; glucose, fructose, sucrose, and starch). Sapwood lipid concentrations were analyzed using the photometric method of Hoch et al. (1999). Briefly, 10-14 mg wood powder was extracted in 1 mL aqueous NaOH for 30 minutes, and glycerol was converted to glycerol-3-phosphate. The amount of liberated glycerol was determined in a 96-well microplate reader at 340 nm (model EL800, BioTek Insturments Inc, Winooski, Vermont, U.S.A.). NSC in the phloem and sapwood was analyzed using a similar photometric method (Hoch et al. 2002). Briefly, 12-14 mg of wood or bark powder was extracted in 1.6 mL distilled water at 100 °C for one hour. An aliquot of this extract was used to determine low molecular weight carbohydrates following enzymatic breakdown of fructose and sucrose to glucose. Enzymatic breakdown of starch to glucose by a fungal amylase (‘Clarase,’ Genencor International Inc., Rochester, New York, U.S.A.) was done using a second aliquot of wood or bark extract. This enzymatic digest occurred at 40 °C overnight. Glucose was converted to gluconate-6-phosphate, and this conversion was measured in a 96-well microplate reader at 340 nm (model EL800, BioTek Insturments Inc., Winooski, Vermont, U.S.A.). Sample nitrogen content was measured using an elemental analyzer (model EA 1110, CE Instruments, Wigan, U.K.) at the University of Montana Environmental Biogeochemistry Laboratory.

Statistical analysis

A one-way ANOVA followed by Tukey's HSD test was used to evaluate differences in phloem lesion length between clones in the fungal inoculation treatment (Figure 2). Bivariate correlations were used to compare phloem lesion length and the percentage of necrotic phloem around the circumference of the tree with initial tree resource concentrations and with the percentage change in tree resource concentrations over time (Figure 3). A general linear model with repeated measures was used to evaluate the effect of treatment on tree resource concentrations over time (Table 2, Figure 4). Sampling date was a repeated measures factor and tree diameter was included as a covariate. Response variables included sapwood lipids, nitrogen, and NSC, and phloem nitrogen and NSC (Table 2). Statistical analyses were performed in PASW Statistics 18 (IBM Statistics).

Results

Tree resistance to *C. polonica* varied between different Norway spruce clones in the fungal inoculation treatment, causing significant differences in phloem lesion lengths between clones (Figure 2). The percentage of necrotic phloem around the circumference of the tree was generally high; seven of nine trees in the fungal inoculation treatment had more than 50 % necrotic phloem, and these trees appeared to be dead or dying one year following treatment (Table 1). In contrast, only three of nine trees attacked by *I. typographus* had > 50 % necrotic phloem, and only two of those trees appeared dead by the following year (Table 1).

Tree susceptibility to *C. polonica* inoculation was not correlated with initial resource concentrations, but was in general negatively correlated with percentage resource change following fungal inoculation (Figure 3). The trees with most necrotic phloem following inoculation had the strongest depletion of phloem NSC from May to August (r = -0.783, p = 0.013), and of phloem NSC and sapwood lipids from May to October (r = -0.875, p = 0.002; r = -0.776, p = 0.014, respectively). The trees with the longest lesions following inoculation had similar negative but non-significant correlations between lesion length and depletion of phloem NSC, sapwood lipids, and sapwood nitrogen from May to August (r = -0.632, p = 0.068; r = -0.622, p = 0.074; r = -0.666, p = 0.050, respectively). The concentration of sapwood lipids continued to decline after August and was negatively correlated with lesion length from May to October (r = -0.784, p = 0.012). Correlations between tree susceptibility and phloem nitrogen or sapwood NSC were not significant.

Treatment (beetle attack, fungal inoculation, control) had some independent effects on tree resource concentrations, and also interacted with sampling date to influence resource concentrations over time (Table 2, Figure 4). A pronounced decline in phloem NSC occurred in the beetle attack and fungal inoculation treatments, with a greater decline occurring in the fungal inoculated trees. This decline occurred entirely between May and August in both treatments. Sapwood NSC concentrations were more variable; beetle attacked trees significantly increased in NSC, but NSC was also initially lower in this treatment. A significant treatment × date interaction occurred for sapwood lipids, which declined consistently from May to October in fungal inoculated trees. No significant changes in nitrogen occurred in any treatment.

Discussion

We studied resource dynamics in Norway spruce (*Picea abies*) to determine whether tree susceptibility to the fungus *Ceratocystis polonica* correlated with initial resource concentrations or resource change over time, and whether resource change over time indicated that *C. polonica* (or other fungi vectored by the spruce bark beetle *Ips typographus*) redistributed stored resources from the sapwood to the phloem of the tree. Our study is the first of its kind to apply control, beetle attack, and fungal inoculation treatments to genetically identical Norway spruce ramets, which allowed us to compare resource concentrations and tree susceptibility without the added variability of genetic differences.

First, we observed significant differences in mean phloem lesion length between clones in the fungal inoculation treatment (Figure 2), suggesting considerable variation in the susceptibility of Norway spruce genotypes to *C. polonica*. High genotypic variation in resistance to *C. polonica* seems to be common in Norway spruce (e.g. Christiansen et al. 1999; Krokene et al. 2003; Zeneli et al. 2006). Smaller phloem lesions or a smaller percentage of necrotic phloem indicate greater tree resistance against fungal pathogens (e.g. Krokene and Solheim 1998, Christiansen et al. 1999; Krokene et al. 2001), and we generally observed higher mortality in trees with larger phloem lesions or more necrotic phloem (Table 1).

Initial tree resource concentrations were unrelated to susceptibility to *C. polonica*, but we observed significant correlations between tree susceptibility and percentage change in resource concentrations over time. A negative correlation occurred between percentage change in phloem NSC and the percentage of necrotic phloem around the tree circumference after fungal inoculation, and a similar, but non-significant correlation occurred between percentage change in NSC and phloem lesion length (Figure 3). This suggests that trees with a greater depletion of phloem NSC were more susceptible to *C. polonica* (Figure 1C). We also observed negative correlations between sapwood nitrogen and lipid depletion and tree susceptibility (Figure 3). Overall, these data and our conceptual model suggest that in more susceptible trees, resources may be depleted by fungal consumption (Figure 1C) instead of tree resistance via allocation to inducible defenses.

We also observed greater declines in phloem non-structural carbohydrates (NSC) and sapwood lipids in trees inoculated with *C. polonica* alone (Figure 4) relative to trees attacked by *I. typographus*. These differences in treatments may have occurred because *I. typographus* can vector other, less pathogenic fungi in addition to *C. polonica*, and the introduction of such fungi could reduce the abundance and pathogenicity of *C. polonica*. However, *I. typographus* predominantly vectors *C. polonica* in this area of southern Norway (Solheim 1991, Krokene and Solheim 1996), and instead, treatment differences were most likely due to above average temperatures in May 2010 that stimulated early beetle emergence and reduced beetle pressure at the time of the fungal inoculation treatment. Indeed, a proportion of the *I. typographus* population had already emerged from hibernation when we placed our pheromone lures, resulting in low and variable attack density on our experimental trees. Trees in the fungal inoculation treatment therefore received considerably more damage than trees in the beetle attack treatment (Table 1), even though fungal inoculation at a density of 400 m^{-2} reflects the natural density of *I. typographus* attacks (Christiansen 1985; Krokene and Solheim 1998). However, research from the North American mountain pine beetle *(Dendroctonus ponderosae*) system also suggests that while beetles and fungi have a mutualistic relationship overall, they probably also compete for resources while in the host tree (E. Lahr and A. Sala, unpublished data). Thus, there is reason to predict that fungal performance might improve in the absence of beetles, as long as the fungal inoculation density is above the threshold required for successfully overcoming tree resistance (Christiansen 1985).

It is clear from our data that beetles do not need to be present for *C. polonica* to have dramatic effects on tree resources, and although we cannot rule out the possibility that *C. polonica* or other associated fungi provide nutritional benefits to *I. typographus*, we did not observe an increase in phloem resource concentrations that would support this hypothesis. Specifically, we were initially surprised not to observe an increase in nitrogen in phloem colonized by fungi (Figure 4) since nitrogen is an important limiting nutrient in insect development (Mattson 1980; White 1993). But, unlike bark beetles that do benefit from fungal-derived nutrients (e.g. Ayres et al. 2001; Bleiker and Six 2007), *I. typographus* has a shorter development time in the tree and overwinters in the ground rather than the host tree. These differences could inherently limit the ability of *C. polonica* or other associated fungi to increase concentrations of limiting nutrients like nitrogen in areas of the tree that would benefit *I. typographus* during its short developmental period in the host tree. However, even without providing direct nutritional benefits, resource uptake by fungi could preclude tree allocation to resistance or enhance fungal growth to such an extent that tree defenses are overwhelmed, or also affect tree function via interruption of water transport (Ballard et al. 1983; Yamoka et al. 1990; Croisé et al. 2001; Kuroda 2001). These possible effects of fungi on the host tree could all indirectly benefit *I. typographus* and deserve further study.

Tree susceptibility to bark beetles and fungi, and the mechanisms that underlie resistance, are the subject of a great deal of research (e.g. reviews by Berryman 1972; Franceschi et al. 2005; Krokene et al. *in press*). Our study demonstrates that information about tree resource dynamics may improve our understanding of conifer susceptibility to fungal pathogens, and may improve our understanding of bark beetle-fungal interactions. While we observed changes in tree stored resource concentrations following fungal inoculation (Table 2, Figure 3), our results do not suggest that *C. polonica* redistributes tree resources in a way that provides nutritional benefits to *I. typographus*. Neither did stored resources appear to benefit the host tree defensively (Figure 1A), despite resource depletion over time (Figure 4). Instead, consistent with our conceptual model (Figure 1C), our data suggest that tree stored resources may benefit fungi. This could indirectly benefit beetles by reducing tree resource allocation to defense or via direct negative effects of fungal growth on tree function. Additional research is needed to further investigate these effects in this and other bark beetle systems. Our conceptual model may be used in other bark beetle systems to help interpret the relationship between tree resource dynamics and susceptibility to fungi and may be particularly useful in understanding susceptibility to and resistance against fungi associated with generalist bark beetles that attack tree species of varying resource quality.

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Table 1. Treatment details and outcome for individual Norway spruce trees.

Treatment	Clone	Diameter (cm)	Inoculations (number)	Necrotic Phloem (%)	Phloem Lesions (cm)[a]	Outcome[b]
Attack	108	26.7	-	35	-	alive
Attack	109	24.0	-	11	-	alive
Attack	113	21.0	-	20	-	alive
Attack	114	27.2	-	32	-	alive
Attack	123	21.2	-	12	-	alive
Attack	124	16.7	-	100	-	dead
Attack	125	17.8	-	31	-	alive
Attack	127	22.3	-	53	-	alive
Attack	129	19.6	-	100	-	dead
Inoculation	108	19.4	293	100	4.5 ± 0.7	dead
Inoculation	109	23.6	355	50	5.0 ± 2.1	alive
Inoculation	113	19.6	295	98	8.0 ± 1.5	dying
Inoculation	114	22.6	341	95	7.9 ± 2.5	dying
Inoculation	123	21.2	319	45	6.5 ± 1.1	alive
Inoculation	124	17.2	259	100	8.3 ± 0.9	dead
Inoculation	125	16.7	252	95	7.0 ± 0.9	dying
Inoculation	127	20.2	305	100	9.1 ± 1.6	dead
Inoculation	129	15.6	235	100	8.8 ± 1.5	dead
Control	108	24.5	-	-	-	alive
Control	109	23.1	-	-	-	alive
Control	113	23.2	-	-	-	alive
Control	114	22.6	-	-	-	alive
Control	123	21.3	-	-	-	alive
Control	124	16.2	-	-	-	alive
Control	125	17.4	-	-	-	alive
Control	127	20.1	-	-	-	alive
Control	129	15.1	-	-	-	alive

[a] Phloem lesion length is mean ± standard deviation.

[b] Outcome is a qualitative assessment of tree health approximately one year following treatment.

Table 2. General linear model with repeated measures showing effects on resource concentrations in Norway spruce sapwood and phloem.

	Sapwood Lipids		Sapwood NSC		Sapwood Nitrogen		Phloem NSC		Phloem Nitrogen	
	F	p	F	p	F	p	F	p	F	p
Intercept	0.105	0.749	4.042	0.056	8.467	0.009	6.055	0.022	41.118	0.000
Treatment	0.888	0.425	12.172	**0.000**	0.007	0.993	10.272	**0.001**	1.379	0.276
Diameter	5.359	**0.030**	4.796	**0.039**	0.102	0.753	0.013	0.909	0.336	0.569
Date	0.579	0.454	0.244	0.786	1.439	0.263	8.538	**0.002**	2.317	0.144
Date × Treatment	9.770	**0.001**	1.411	0.236	2.314	0.095	3.328	**0.018**	1.526	0.243
Date × Diameter	0.617	0.440	0.366	0.697	1.424	0.267	5.396	**0.012**	1.920	0.182

Treatment (control, beetle attack, fungal inoculation) is a factor, sampling date is a repeated measures factor, and tree diameter is a covariate. Bold values indicate significant effects.

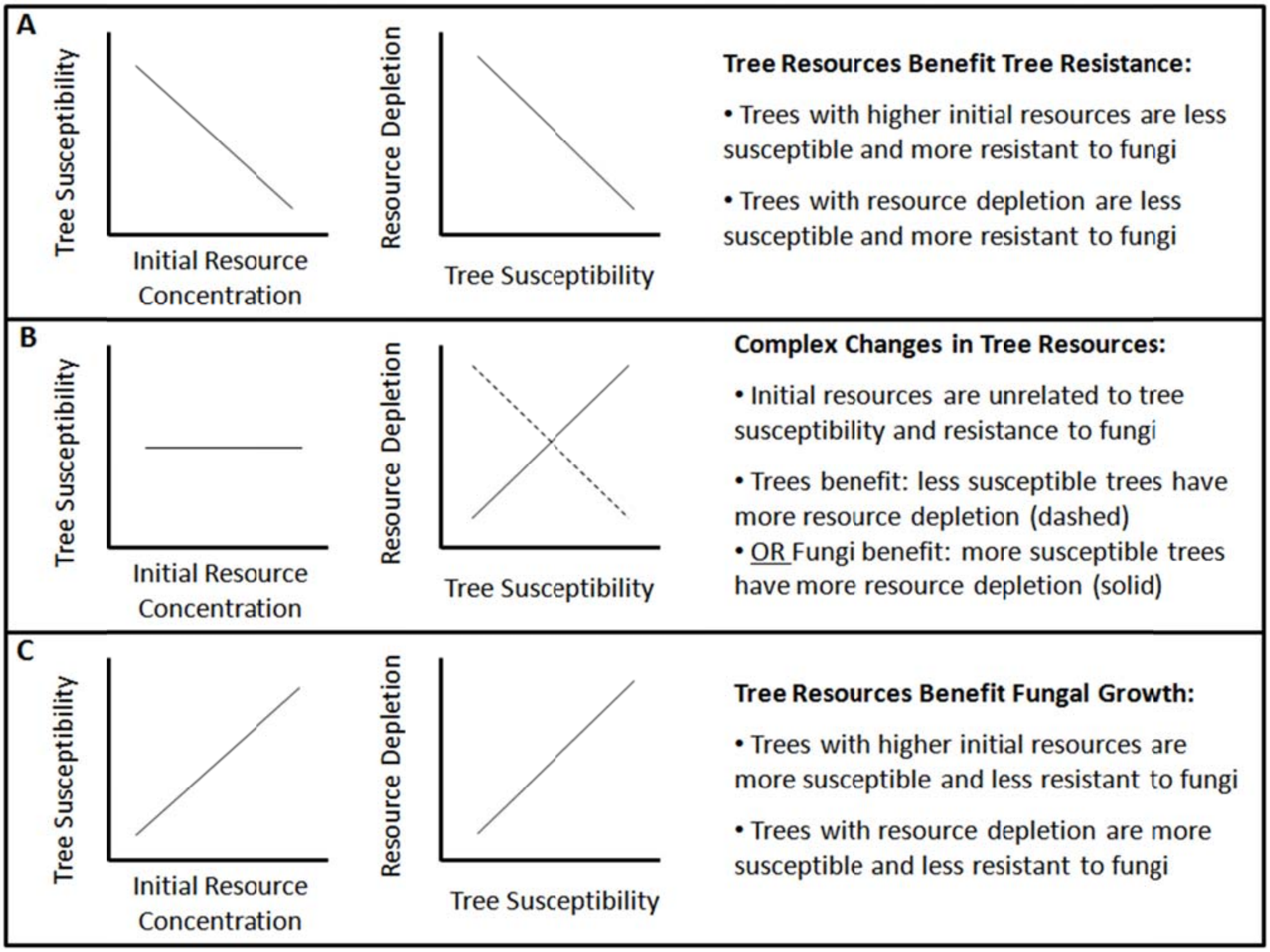

Figure 1. Conceptual model illustrating three possible changes in tree resources in relation to tree susceptibility to fungal colonization. (A) If stored resources are overall more beneficial to the tree than to the fungus, trees that have higher initial resource concentrations and resource depletion over time (a negative change in resources) will be less susceptible and more resistant to fungal colonization. (B) Tree susceptibility may be independent of initial resource concentrations and tree susceptibility may cause complex changes in tree resources over time. For example, independent of initial resource concentrations, less susceptible trees may have more resource depletion and successfully resist fungal colonization (dashed line). Alternatively, independent of initial resource concentrations, more susceptible trees may have more resource depletion because

they are less resistant to fungal colonization and fungi consume resources (solid line). (C) If stored resources are overall more beneficial to the fungus than to the tree, trees with higher initial resource concentrations and resource depletion over time will be more susceptible and less resistant to fungal colonization. This could occur if high initial resource concentrations benefit fungi regardless of tree resistance, if resource consumption enhances fungal growth to such an extent that tree resistance breaks down, or if fungal resource consumption prevents tree resistance.

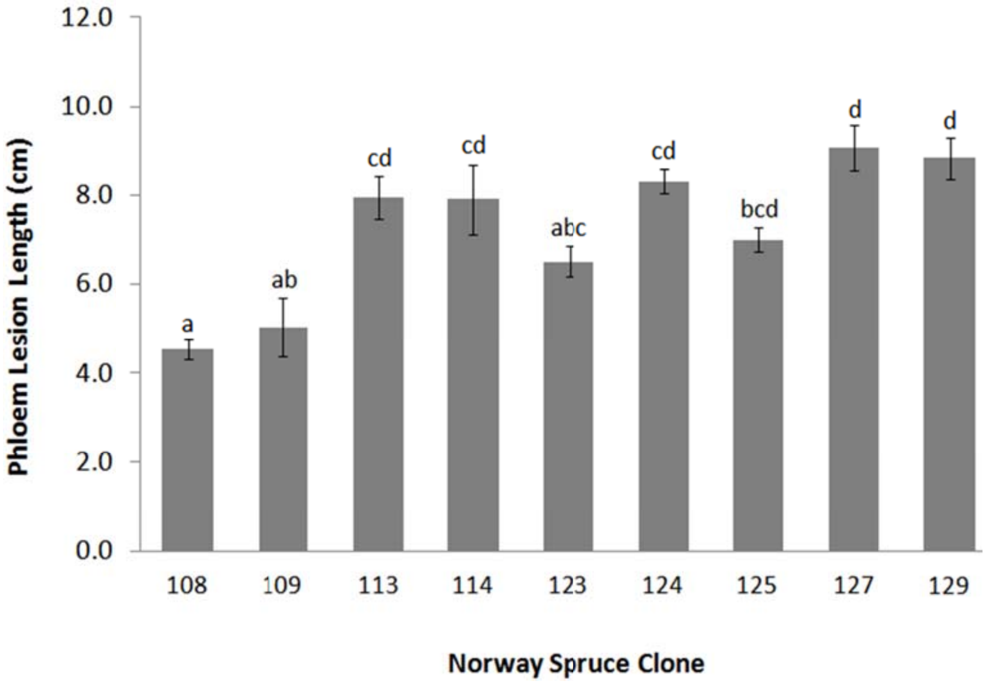

Figure 2. Mean phloem lesion length for Norway spruce clones inoculated with the fungus *Ceratocystis polonica*. Error bars show ± 1 standard error. Letters indicate significant differences between clones. Further details about the different clones are given in Table 1.

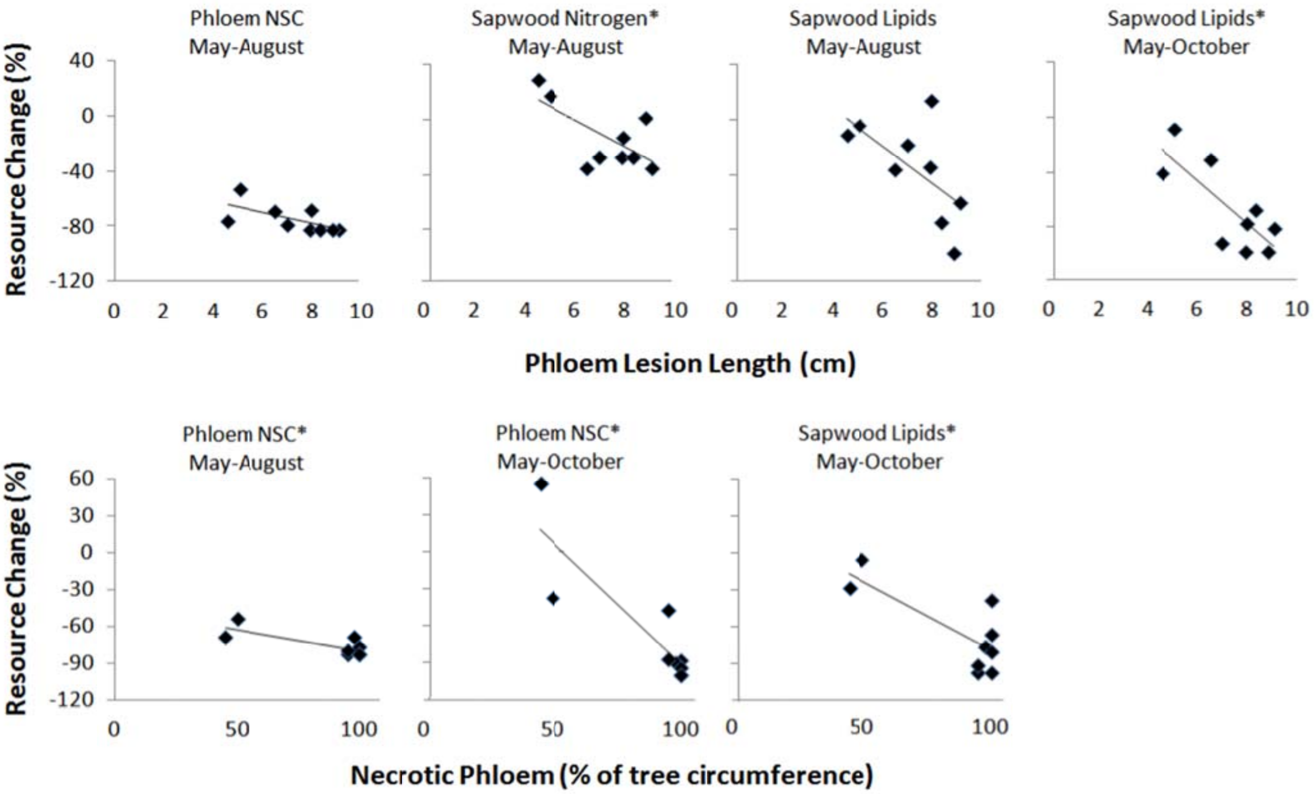

Figure 3. Correlations between Norway spruce resistance to the fungus *Ceratocystis polonica* and percentage change in non-structural carbohydrate (NSC), nitrogen, and lipids over time. Tree resistance was measured as phloem lesion length (upper panels) or as the percentage of necrotic phloem around the circumference of the tree (lower panels). An asterisk (*) denotes significant correlations ($p < 0.05$), as described in the Results.

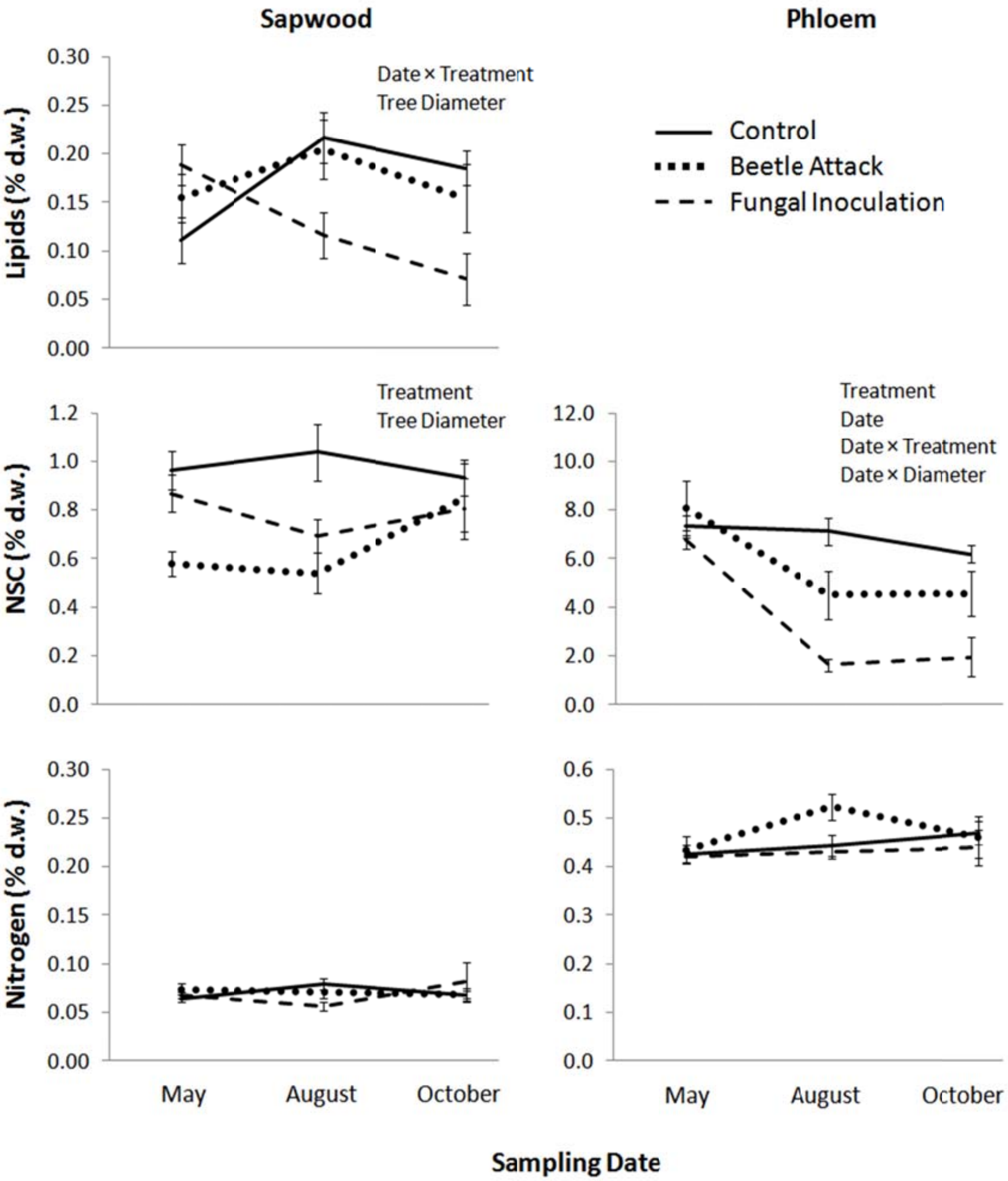

Figure 4. Change in lipids, non-structural carbohydrates (NSC), and nitrogen over time in the sapwood and phloem of control (solid lines), beetle attacked (short dashed lines), and fungal inoculated Norway spruce trees (long dashed lines). Error bars show ± 1 standard error. Significant ANOVA effects are indicated in the upper right of each panel. Note differences in y-axis scale between the sapwood and phloem for NSC and nitrogen.