

Potential Host Use by the Mediterranean Pine Engraver on Novel Tree Species

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Abstract

Introduced species have the potential to cause great environmental damage, but many species introduced to an area will not have a large impact. It is critical that we develop the ability to predict which species will become damaging invaders, and manage ecosystems to minimize the probability that introduced species could establish. I present research results on the potential of the Mediterranean pine engraver, *Orthotomicus erosus* (Wollaston), to utilize North American tree species as hosts. I confirm that several North American conifers, species of pine and spruce, are suitable for the development of *O. erosus*. I then investigate the host acceptance behaviors of adults in contact with the outer bark. I show that differences among species occur during boring into the outer bark, and that beetles bore into both suitable and unsuitable tree species. The behavior of beetles before they are in contact with the bark was investigated with an olfactometer; odors from several conifer species do not affect the behavior of adult beetles. I also test the feeding response of beetles to extracts of bark and phloem chemicals. Several extracts contain feeding incitant or stimulant compounds, but the presence of these compounds in various tree species is different than adult bark boring behavior on those species. Finally, I used the data from my previous experiments and the phylogenetic distance among tree species in an attempt to predict the response of *O. erosus* to a second set of tree species from independent validation experiments. Phylogenetic distance from species with known beetle responses was not a good predictor of beetle response to novel plants. This body of work shows that *O. erosus* may accept a larger set of trees than are suitable for reproduction. Furthermore, adult host acceptance behavior is not determined

solely by gustatory stimuli. Detailed knowledge about the stimuli governing host acceptance behavior is needed to predict the acceptability of new tree species to the beetle. The presence of acceptable but developmentally unsuitable plants may determine whether species such as *O. erosus* are able to establish in a new environment.

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Preface

This dissertation is formatted as a series of manuscripts for submission to scientific journals. There are slight differences in the formatting of the chapters depending on the target journal. Chapter two, Acceptance and suitability of novel North American hosts for *Orthotomicus erosus*, a potential invasive bark beetle, is formatted for *Biological Invasions*; authors for Chapter two are Abigail J. Walter, Robert C. Venette, and Stephen A. Kells. Chapter three, Walking response of Mediterranean pine engraver, *Orthotomicus erosus*, to host and non-host volatiles in a laboratory olfactometer, is formatted for the *Journal of Insect Behavior*; authors for Chapter three are A.J. Walter, R.C. Venette, S.A. Kells, and Steven J. Seybold. Chapter four, Allopatric host and non-host plants contain feeding stimulants for the Mediterranean pine engraver, *Orthotomicus erosus* (Coleoptera: Scolytidae), is formatted for *Chemoecology*; A.J. Walter, R.C. Venette, S.A. Kells, and S.J. Seybold are authors for chapter four. Finally, chapter five, Development and host selection behaviors by *Orthotomicus erosus* on allopatric plants are not correlated with plant phylogeny, is formatted for *Ecological Entomology*; A.J. Walter, R.C. Venette, S.A. Kells, Jana C. Lee, and S.J. Seybold are authors for chapter five.

Voucher specimens from these experiments are deposited in the University of Minnesota Insect Museum.

Chapter 1: Host use in invasive insects and *Orhotomicus erosus* as a model system

The ability of an insect to select an oviposition site and the ability of the insect's offspring to use the resources present at the oviposition site are major determinants of fitness. The choice of oviposition site, especially for those species where the larvae are relatively sessile, has profound impacts on the survival and potential fecundity of the next generation. Offspring survivorship is a major determinant of insect population dynamics and whether a particular species attains pest status. Understanding host utilization is thus critical to predicting the dynamics of insect populations that are of interest to humans and may lead to novel control methods (Kennedy 1965). In the case of potential invasive insects, understanding host utilization can improve our understanding of the risk associated with a particular species and our methods for predicting of the consequences of an introduction.

The host selection process

What is a host?

The host range of an herbivorous insect is often defined as the list of plant species that an insect has been observed to associate with in nature (Schaffner 2001). Within this broad definition, many distinctions can be made based on, for example, whether a particular plant species is nutritionally complete for larval development or whether the plant is typically encountered by the insect (Table 1.1). Different authors have sometimes used different terms to define the same concept, for example the physiological host range of Schaffner (2001) is equivalent to the food plant range of Schoonhoven et al. (1998) and the potential host plant range of the larvae of Wiklund (1975).

Despite the plethora of host-type definitions available in the literature, 'host' itself is seldom explicitly defined. Host use, however, is defined as the 'completed life cycle' of an insect on a plant species (Pemberton 2000). Therefore, I define a host of an herbivorous insect as an individual plant that provides adequate resources for all stages of the life cycle of an insect, elicits all necessary behaviors in order for the life cycle to be completed, and lacks factors (i.e., toxins and deterrents) that would prevent the completion of the life cycle through physiological or behavioral effects. For insect species that utilize more than one plant species during their lifetime or seasonal population cycle, a host would meet the above criteria for the relevant stages or time frame.

Components of host use

A potential host must necessarily possess several characteristics to allow an insect to complete its life cycle. These plant characteristics can be divided into two classes with importance for insects: 1) acceptability and 2) suitability. Acceptability is defined as the set of traits that determine whether a plant is selected for feeding or oviposition (Schoonhoven et al. 2005), and may be considered the set of plant characteristics that influence an insect's behavior before it has made an investment (i.e. feeding or oviposition) in the plant. Suitability is the set of traits determining that influence the physiology and behavior of an insect on a host (adapted from a definition for insect parasitoids, Vinson and Iwantsch 1980), and may be considered the set of plant characteristics that influence an insect's performance after it has made an investment in the plant.

Plant acceptance

Plant acceptance occurs when an insect sustains feeding or oviposits on a plant; several behavioral steps may be necessary for acceptance to take place. Acceptance does not imply that any consideration of alternatives has taken place (Schoonhoven et al. 2005). In contrast, both selection (to choose from alternatives based on distinct characteristics) and preference (selection more often than would be expected by chance) imply that at least two possible options have been considered (Miller and Strickler 1984, Schoonhoven et al. 2005). No-choice laboratory assay (Barton Browne and Withers 2002) or host utilization in the midst of a monoculture (Schoonhoven et al. 2005) are by definition both cases where host acceptance takes place without host selection or preference.

There are several steps necessary for host acceptance to occur (see Figure 1.1 for an example for bark beetles). An insect that is (presumably) not in contact with a plant must become responsive to plant cues, receive the appropriate cues, come into contact with the plant, and realize that the plant is an appropriate host. These steps have been defined as arousal, encounter, finding, and recognition, respectively (Table 1.2). These behaviors may be active or passive, and different terminology has sometimes been used for active and passive behaviors leading to host acceptance. Host acceptance may occur through action chains, ‘predictable sequences of behavioral acts’ by the insect where each behavior is governed by a stimulus or set of stimuli (Schoonhoven et al. 2005).

In order for host acceptance behaviors to occur, the insect must be within the active space, the physical space ‘within which the intensity of a stimulus or cue is above the threshold for a behavioral response’ of plant stimuli (Schoonhoven et al. 2005).

Different types of stimuli have different effects in eliciting or discouraging insect host acceptance behaviors. For stimuli encountered before the insect is in physical contact with the plant, I use the terminology of Dethier et al. (1960) (Table 1.3), whereas for stimuli that affect feeding behaviors, I use the terminology of Beck (1965) (Table 1.4). Where Dethier and Beck considered only chemicals as possible behavioral stimulants, I include any cue perceivable by the insect (visual, olfactory, gustatory, etc.).

Plant Suitability

Suitability is a measure of how a plant affects the fitness of an insect. Suitability may include measures such as survival, reproduction, and development-time. The host plant may also affect insect characteristics that are important determinants of fitness in certain environments. For example, the consumption of a particular plant is also known to influence immune response (Ojala et al. 2005) and nutrition affects insect cold-hardiness (Lee 1991). Such responses to environmental stress are components of host suitability if the stress affects insect fitness in the habitat where the insect is located.

Patterns in host suitability and acceptability

When adult insects make host selection decisions for their sedentary larvae, ‘there should be strong selective forces affecting such decisions in the parent, which, under the constraints imposed by the evolutionary process, the natural environment, and the construction of the organism, will maximize total fitness returns from the offspring (Mayhew 1997).’

This prediction is termed optimal oviposition or the preference-performance hypothesis (Wiklund 1975, Jermy 1984, Scheirs and De Bruyn 2002). In a meta-analysis that tested the preference-performance hypothesis, 78 of 135 studies supported the hypothesis, 28 partially agreed with it, and 34 contradicted it. The frequency of negative results was probably higher than they encountered in the literature (Mayhew 1997).

Indeed, ‘it is not uncommon to find a mismatch between oviposition preferences and hierarchies of host suitability for larval performance, with examples from a variety of insect systems (Stastny et al. 2006).’ There are several reasons why a mismatch between adult behavior and larval performance could occur, even if the optimal host plants were accepted by adults when they encountered them: 1) the phenology of the insect and plant may make the best host plants unavailable for oviposition; 2) female foraging may not take place in the same habitat as the optimal hosts; 3) optimal hosts may be less abundant than suboptimal hosts; and 4) resources necessary for adults may be located far away from optimal host plants for the larvae (Schoonhoven et al. 1998). In addition, plants may offer resources such as predator free space that are often not considered in measures of offspring performance (Bernays and Minkenberg 1997, Ishihara and Ohgushi 2008).

Another reason for mismatches between adult preference and larval performance is that selection should act to widen the host range of sedentary larvae, so that they could consume whatever plant they find themselves on, while narrowing the host range of the adults so that only the best host plants are selected. Thus, in an evolutionarily stable state, the set of plants palatable to a larva should be larger than the set of plants used for oviposition by the adult (Wiklund 1975). The selection pressures on offspring and adults can lead to differing patterns of adult preference and larval performance because adult

host selection behaviors and larval development are controlled by different sets of genes (Wiklund 1975, Thompson et al. 1990, Janz 2002). Cases where larvae are able to use a wider set of plants than are selected by adults are known from herbivorous Hymenoptera (Roininen and Tahvanainen 1989), Lepidoptera (Wiklund 1975, Janz et al. 2001), Diptera (Gratton and Welter 1998), and Coleoptera (the bark beetles) (Sauvard 2004). An important consequence of the common pattern of a wider fundamental host range than realized host range is that adult behavior is an important determinant of host use for many species (Bernays and Chapman 1994, Sauvard 2004, Schoonhoven et al. 2005), and behavior must be understood to forecast host use in the field (Courtney and Kibota 1990).

Despite the strong selective pressures for adult host range to encompass those plants suitable for larvae or a subset of the suitable plants, a number of instances where adults select plants for oviposition that do not support the development of larvae are known from Lepidoptera (Straatman 1962, Chew 1977, Copp and Davenport 1978, Rodman and Chew 1980, Graves and Shapiro 2003, Badenes-Perez et al. 2006, Casagrande and Dacey 2007), Hymenoptera (Barre et al. 2002), and Diptera (Harris et al. 2001). In all of these cases, the plant has been introduced. It is reasonable to assume that similar cases occur when an insect has been introduced, but they have not been reported.

Host utilization by newly introduced species

Host recognition of novel plant species by insects

When an herbivorous insect first enters a new geographic area, it may encounter plant species with which it has no recent evolutionary history of association. If the insect

population is able to persist in the new environment, selective forces should refine the responses of the insect to novel plant species. However, in order for populations to persist reproduction must occur; utilization of acceptable and suitable hosts before the insect has adapted to its new environment is absolutely critical to the success of the invasion (Payne et al. 2004). In this context, host recognition will take place based on the similarity of the stimuli presented by plants in the new environment to the insects' neural image of a host, which need not be based on experience (Dethier 1982).

Acceptance of a suitable host will take place when the insect is 'pre-adapted' to recognize novel host and reject novel non-host plants (Jermy 1988). This is known as ecological fitting, the occurrence of genotypes that are 'serendipitously robust with respect to the challenges of living' in a new geographic area (Janzen 1985). 'Ecological fitting may facilitate the formation of novel species associations to a much greater extent than that allowed if mutations and changes in gene frequencies were a pre-requisite for their formation (Agosta 2006),' and can be expected to enable host range expansion during biological invasions (Brooks et al. 2006). The addition of species to the realized host range of an organism, termed host range expansion, can occur without genetic changes in either acceptance or performance of the organism (Schaffner 2001).

When new species associations arise and ecological fitting does not occur, a newly introduced insect may accept unsuitable plants or fail to accept suitable plants (Jaenike 1990, Liebert and Starks 2004). If the insect fails to accept suitable plants, reproduction will not occur and the population will go extinct. Alternatively, if the species accepts unsuitable plants, the impact on the population will depend on the rates at which suitable and unsuitable plants are accepted. The introduction of an acceptable but

unsuitable snail species has been suggested for the biological control of *Schistosoma haematobium*, a human parasite that uses snails as an intermediate host (Allan et al. 2009). The term ‘evolutionary trap’ has been used to describe introduced plants that are acceptable to an insect species but unsuitable for its development (Schlaepfer et al. 2005), but I disagree with this usage because these associations arise without evolutionary changes to either species.

Declining acceptance thresholds

A number of authors (Thorsteinson 1960, Beck 1965, Dethier 1982, Miller and Strickler 1984, Courtney et al. 1989, Bell 1990, Courtney and Kibota 1990) have advanced models of insect host selection where the probability that an insect will accept a plant changes depending on the internal state of the insect (i.e., changes in egg load or starvation). This situation is known to be the case for bark beetles, which are often reported not to be host responsive until they have flown or been starved for a period of time (Sauvard 2004). A key prediction of these conceptual models is that the threshold for host acceptance will decline in deprived insects (i.e. insects will more readily accept suboptimal hosts when they have been denied hosts). One of the more general statements of this conceptual model is the hierarchy-threshold model (Courtney et al. 1989), which states that ‘insects may, at different points in their lives, be specialized on high-ranking hosts, or accept a wide range of hosts, both low- and high-ranking. They should not, however, become specialized on previously low-ranking hosts’ (Courtney et al. 1989).

The hierarchy-threshold model predicts that plant species will always present the same level of stimulation. An insects ranked preference for these plants is determined by

positive and negative stimuli presented to the insect. The insect's threshold for accepting the plants, its motivation, will vary in accord with its internal state. For example, a replete insect carrying only a few eggs may accept only hosts of very high rank, or no hosts at all, for oviposition. As the internal physiology changes, for examples as the egg load increases, the insect will become more motivated to oviposit, and plants of lower rank will be accepted. This model assumes that plants are encountered by insects one at a time (no memory is carried from one encounter to the next) and by chance, so the insect's behavior is acceptance rather than selection. The model also predicts that an insect with a threshold such that it will accept several plants will oviposit on the first encountered plant whose stimuli exceed its acceptance threshold, regardless of the ranking of the encountered plant relative to other plants in the surrounding area.

The loosening of host acceptance thresholds in deprived insects may be important in biological invasions. Insects with low motivation to oviposit may accept only highly ranked plants. If the set of stimuli of highest ranking closely matches the host(s) that an insect uses in its native environment (a normal host), and these plants are not present, no plants may be accepted at first. However, as motivation increases in the absence of the normal host, the threshold for acceptance will drop. Since it can reasonably be assumed that at least some individuals will attempt to feed or oviposit on a plant before they die of starvation or exposure, this means that insects will attempt to use plants in the new environment which which they have no history of association (a novel host). Successful insect reproduction will then depend on whether the highest ranking species in the new environment were also suitable for the larvae.

Host selection during a biological invasion

When a population of insects first enters a new environment, they may be able to persist because of ecological fitting. The internal state of the insects are likely to change over time until they are ready to utilize a host. If arousal depends on the internal state of the insect, host search will commence at this point. However, if arousal depends on an external stimulus, host search will not commence unless that stimulus is present. For example, the appearance of host switching morphs of aphids may depend on day length cues (Dixon 1998).

Prior to host acceptance, the insect will move around the environment, encountering stimuli from the plants in it. When relevant stimuli from a plant exceed the internal threshold of the insect, the insect will progress to the next behavioral stage in the host selection action chain (Figure 1.1). The various behaviors may involve active or passive actions with respect to the plant emitting the stimulus, and have evolved such that they would increase the probability that the insect would encounter stimuli that would trigger it to move to the next behavior in the action chain in the insect's native environment. For instance, searching insects may fly at a constant angle to the wind. Olfactory cues might lead them to increase their turning radius in an area, increasing the probability of coming into physical contact with a plant (Schoonhoven et al. 1998). Once the insect is in physical contact with the plant, surface texture might elicit biting, and chemical cues might trigger feeding or oviposition. The insect may be able to complete the host acceptance action chain on suitable or unsuitable plants.

Once host acceptance has occurred, the suitability of the plant will determine whether the insect is able to successfully reproduce. If at least some of the population

accepts suitable plants, another generation will occur, and the process begins again. However if no members of the population accept suitable plants, either because they are not present in the environment, are unavailable due to their phenology, or because unsuitable plants are higher ranked, the population could go extinct. Extinction of a population due to acceptance of unsuitable plants has not been reported, but is most likely to occur following an introduction of an herbivore. Failed biological invasion are 'notoriously difficult' to observe and document (Payne et al. 2004).

***Orthotomicus erosus*: A model system**

Life history and distribution

The Mediterranean pine engraver, *Orthotomicus erosus* (Wollaston) (Coleoptera: Scolytidae), is a Eurasian bark beetle. It is a multivoltine species, with six or seven generations per year reported in Israel (Mendel 1983, Mendel et al. 1985) and three or four generations per year reported in the central valley of California (Lee et al. 2007). In California, adults fly year-round except during late December and January, and eggs have been observed between the first week of March and the last week of November (Lee et al. 2007). Flight activity by overwintering beetles can occur when the temperature exceeds 12°C; during summer, flight occurs when the temperature exceeds 21°C (Mendel et al. 1991). Flight does not occur at temperatures above 34°C (Mendel et al. 1991).

Mendel and Halperin (Mendel and Halperin 1982) describe the basic life history of *O. erosus*. An infestation is initiated when a male locates a host, bores through the bark of the tree and into the phloem, and constructs a nuptial chamber. He is joined by

one to three females who mate with the male and construct egg galleries parallel to the vertical axis of the tree. When two females are present, they construct their galleries in opposite directions from the nuptial chamber. If three females are present, two of the galleries will be constructed in the same direction, so that the three egg galleries together resemble a Y. The females oviposit on alternating sides of the egg galleries, although if the female detects another egg gallery close by, all of the eggs may be deposited on the side of the egg gallery farthest away from the neighboring gallery. The larvae bore outward, each larvae in its own gallery. The average length of egg galleries is 35-71 mm, and the average number of eggs deposited in each egg gallery is 26-75.

Orthotomicus erosus develops through 3 larval instars. The lower threshold for development is 13.6°C, and the upper threshold is near 40°C. Oviposition takes place when temperatures are between 18 and 42°C (Mendel and Halperin 1982). The first brood adults begin to emerge from the host tree at 326-383 degree days after the parent female enters the bark (base temperature 25°C, 95% confidence interval calculated from data in Mendel and Halperin 1982 by R.C. Venette, personal communication). In a laboratory rearing study, the first exit holes of a brood on *Pinus taeda* were observed after 523.5 (threshold 13.6-39°C) or 334.7 (threshold 18-39°C) degree days (Lee et al. 2008).

Larvae feed on the phloem and etch the outer xylem of the host tree, and the pupal chamber is constructed here as well. When the adult emerges, it will continue to feed in the deteriorating phloem for maturation feeding if the bark is sufficiently moist. If the bark is too dry or when maturation feeding is complete, the insect emerges from the host

tree to search for a new host for further maturation feeding or mating (Mendel and Halperin 1982).

All life stages except for eggs have been reported to overwinter (Mendel 1983, Lee et al. 2007). All life stages appear to be freeze intolerant, but they are chill tolerant. The adults are capable of cold acclimation; the mean supercooling point of cold acclimated adults is -22°C , and the lower lethal temperature of adults is very near this value (Venette et al. 2008). Adult beetles may emerge from their natal host prior to overwintering and form aggregations of a few to several hundred individual under the bark of host or non-host trees (Mendel 1983, Mendel et al. 1985).

A number of other organisms are associated with *O. erosus*. These include the nematode *Bursaphelenchus fungivorus* (Arias et al. 2005), and the fungi *Ophiostoma (Ceratocystis) ips*, *Leptographium lundbergii*, *L. serpens*, and *Graphium pseudomiticum*. *Ophiostoma ips* has been isolated from *Orthotomicus erosus* infesting cut pine in California (Lee et al. 2007).

The distribution of *O. erosus* includes the Mediterranean basin, Central Europe, the Iberian Peninsula, the Canary Islands, the country of Georgia, the United Kingdom, Turkey, Iran, and Northern Africa (Mendel and Halperin 1982, Mendel 1988, Arias et al. 2005). The beetle has been introduced to a number of areas and has adventive ranges in South Africa, Tajikistan, and Chile (Wood and Bright 1992, Eglitis 2000). The pest has been introduced into California, where there is an established population in the Central Valley (Lee et al. 2007) (Table 1.5).

Like many bark beetles, *O. erosus* employs a pheromone system to control aggregation onto host material. The major components of the aggregation pheromone

have been reported as 2—methyl—3—buten—2—ol and 2—methy—6—methylene--2,7—octadien—4—el (ipsdienol) (Giesen et al. 1984), An effective attractant for California populations of *O. erosus* is comprised of (-)ipsdienol and 2—methyl—3—buten—2—ol, and the host compound α —pinene (Seybold and Downing 2009). The addition of amitinol, (+)-ipsdienol, (-)—*cis*—verbenol, or verbenone decreases the attractiveness of a lure (Kohnle et al. 1988, Paiva et al. 1988, Seybold et al. 2006).

Host range

The complete host range of *O. erosus* is not clear. It includes many species in the genus *Pinus* (Mendel and Halperin 1982, Wood and Bright 1992, Arias et al. 2005), and it may also include members of the genera *Pseudotsuga*, *Picea*, *Abies*, and *Cedrus* within the family Pinaceae (Wood and Bright 1992) and the genus *Cupressus* within the family Cupressaceae (Mendel and Halperin 1982, Lee et al. 2008) (Table 1.6). Some of the reported hosts may actually be used for overwintering rather than oviposition or may not support complete development of the larvae (Mendel and Halperin 1982). In cage studies in its native range, *O. erosus* accepted four pine species, two spruces, and two firs indiscriminately, though all those trees were accepted more often than a sympatric cypress (Chararas et al. 1982).

Despite the wide reported host range, the host use potential of *Orthotomicus erosus* has not been extensively studied until recently. Host suitability testing in California has revealed that many pines (*Pinus* sp.), Douglas—fir, *Pseudotsuga menziesii*, and at least two spruces, *Picea glauca* (Moench) Voss and *Pic. mariana* (Mill.) Britton, Sterns, and

Poggenb. (Pinaceae) (Lee et al. 2008) are suitable for the beetle. Several stimuli of host and non-host trees are known to affect host-acceptance behaviors of *O. erosus*. (5*S*,7*S*)-(E)-conophthorin, a volatile common to several angiosperm trees, is antennally active for *O. erosus* (Huber et al. 2000) and can function as a potent interruptant for some bark beetles (Graves et al. 2008). Conophthorin has been reported to inhibit the response of *O. erosus* to pheromone lures (Zhang et al. 2002), although this effect was not observed in other experiments (Zhang and Schlyter 2004, S.J. Seybold, personal communication). Green leaf volatiles have also been reported to interfere with the attraction of *O. erosus* to pheromone lures (Zhang and Schlyter 2004). A component of the aggregation pheromone of *O. erosus*, 2-methyl-3-buten-2-ol, is known from ten species of North American pines, and may affect the attraction of the beetle to these species (Seybold et al. 2006).

Pest status

Orthotomicus erosus is considered one of the principal bark beetle pests of managed pine plantations in Israel (Mendel et al. 1985) and northern Iran (Mendel 1988). It also caused economic losses on pine plantations where it was introduced into the Southern Hemisphere (Lee et al. 2007). *Orthotomicus erosus* usually attacks stressed or wounded trees over five years old, although when the beetle population reaches outbreak level, healthy trees may be attacked and killed (Mendel et al. 1985, Arias et al. 2005). Outbreaks occur when populations build as a result of the presence of a large number of disturbed, weakened hosts, for example following thinning, pruning, drought, or fire in a nearby area (Mendel 1983, Mendel et al. 1991). *Orthotomicus erosus* is commonly found

in association with other bark beetles (Eglitis 2000), and its niche breadth (defined as bark thickness classes on infested logs) is wider when other species of bark beetles are present (Amezaga and Rodriguez 1998).

Risk to North America

Of the woodboring insects recently reported in the western United States ‘*O. erosus* has perhaps the most potential to have a strong impact on conifers in western U.S. forests and elsewhere in North America (Seybold and Downing 2009).’ The appearance of *O. erosus* may necessitate changes in management of many urban and peri-urban forests (Seybold and Downing 2009).

In an assessment of the risk posed by *O. erosus* to North America, the beetle was considered a very high risk, but with a high degree of uncertainty surrounding the assessment (Eglitis 2000). The beetle is a high risk to cause economic damage in the U.S. because it attacks trees with high commercial value and may decrease their value or cause loss of markets due to quarantine restrictions and it may kill trees, predispose trees to attack, or vector tree killing organisms. *O. erosus* has a high risk rating for spread after introduction because of its cryptic nature, potentially broad host range, the contiguous distribution of potential hosts, and its potential to be redistributed by humans moving wood products. The risk of establishment by *O. erosus* is high because suitable climatic conditions and hosts exist near points of probable introduction, it has a high reproductive potential, it has directed host-search, and it has successfully established in other parts of the world and on novel host plants.

Arrival in North America

Orthotomicus erosus is one of the most frequently intercepted exotic Scolytids at U.S. ports of entry (Brockhoff et al. 2006b, Haack 2006). Frequent introduction greatly increases the probability that the beetle will establish in the U.S. (Williamson 1996). The first established U.S. populations of the beetle were reported in 2004 after *O. erosus* was detected in traps at the Fresno Zoo (Lee et al. 2007), although the beetle had likely been present since at least 2001 (Seybold and Downing 2009). Currently, the beetle occurs in at least ten counties in California (Seybold and Downing 2009).

Dissertation Goals and Objectives

This dissertation is comprised of a series of experiments aimed at improved forecasting of the potential host use by *Orthotomicus erosus* on North American tree species. The experiments were designed to: 1) describe the acceptability and suitability of a set of North American species to *O. erosus*; 2) determine whether chemicals encountered by beetles boring in the bark and phloem of those species were major determinants of this acceptability; and 3) determine whether knowledge of the phylogeny of the potential host species could be used to accurately predict acceptability and suitability. The following section outlines each dissertation chapter.

Chapter 2: Acceptability and suitability of novel North American hosts for *Orthotomicus erosus*, a potential invasive bark beetle. *Orthotomicus erosus* has been reported in association with a wide range of host plants in its native and adventive range

(reviewed in Lee et al. 2008, Walter et al. 2008). This chapter 1) describes the suitability of six North American tree species in terms of reproduction when adults were forced to colonize cut logs, and 2) describes the behavior of adult beetles confined on the outer bark of the tree species. I tested the hypothesis that when confronted with novel hosts, the adult beetles will enter the outer bark and bore in the phloem of those tree species that support the development of their larvae, but not those that do not support larval development.

Chapter 3: **Walking response of the Mediterranean pine engraver, *Orthotomicus erosus*, to host and non-host volatiles in a laboratory olfactometer.**

This chapter extends the work in Chapter 2 by describing the behavior of *O. erosus* when host odors are encountered before the insect is in physical contact with the tree. I used a new olfactometer design in this laboratory research. These studies address two hypotheses: 1) odors from selected tree species elicit a behavioral response from *O. erosus*; and 2) odors from trees that differ in their suitability or short-range acceptability also differ in their attractiveness to *O. erosus*.

Chapter 4: **Allopatric host and non-host plants contain feedants for *Orthotomicus erosus* (Coleoptera: Scolytidae).** The behavior of beetles boring into the outer bark appears to be the major determinant of plant acceptability for *O. erosus*. In this chapter, I describe the behavior of beetles presented with chemicals extracted from the outer bark and phloem of four North American tree species applied to a neutral substrate. I hypothesized that the species where the beetle has the highest probability of

boring should contain chemicals with feedant (incitant or stimulant) effects, and species where the beetle has low boring activity would contain antifeedant (suppressant or deterrent) compounds.

Chapter 5: Plant phylogeny is a poor determinant of host range for an oligophagous insect invader. If the genetic relatedness of plants reflects their similarity of their acceptability and/or suitability for an insect, plant phylogeny could be used to make host use predictions. I develop a set of regression models for acceptability characteristics and tree suitability based on phylogenetic distances between tree genera, evaluate the significance of the models, and test the predictions of the models against independent validation datasets. I hypothesized that the behavior and development potential of *O. erosus* on different tree species should be related to the phylogenetic distance among plants, and that the relationship between beetle response and genetic distance can be used to predict the response of an insect to novel species.

Finally, Chapter 6: **Steps Towards Predicting and Preventing Biological Invasions.** In this epilogue, I discuss the implications of the results I obtained in previous chapters for invasive species management. Detailed biological knowledge may be necessary to predict the behavior of individual species in new environments. However, if herbivores have a tendency to accept some unsuitable species in novel environments, then managing ecosystems to increase native plant diversity may make them less prone to invasion.

Table 1.1 Host range definitions.

Term	Definition	Reference
Physiological host range	The set of plants ‘on which the larvae can complete their development’, may also be called fundamental host range of larval development or larval host range	Schaffner 2001
Food plant range	Equivalent to physiological host range	Schoonhoven et al. 1998
Potential host plant range of the larvae	Equivalent to physiological host range	Wiklund 1975
Fundamental host range	The set of plants ‘on which the organism can complete its life cycle’, may be divided by insect stage, i.e., the fundamental host range of larval development, the fundamental host range of female oviposition behavior	Schaffner 2001
Realized host range	The set of plants that ‘a species uses under natural conditions’, a subset of the fundamental host range based on geographic or behavioral filters	Schaffner 2001
Natural host plant range of the adults	The set of plant species used for oviposition in nature	Wiklund 1975
Potential host plant range of the adults	The set of plant species which are used for oviposition when encountered	Wiklund 1975
Natural host plant range of the larvae	The set of plant species fed on by the larvae in nature	Wiklund 1975

Table 1.2. Definitions of possible components of host plant acceptance.

Term	Definition	Reference
Arousal	The transition of an unresponsive insect to a state where it is ready to respond to host stimuli	Bernays and Chapman 1994
Host searching	The process whereby an insect far away from a plant comes into contact with or proximity to it; does not denote directionality in movement	Schoonhoven et al. 2005
Host pursuit	'Movements stimulated by cues from the resource that increase the probability of establishing and maintaining contact with the source'	Miller and Strickler 1984
Encounter	'The point where sensory information from the resource is first received,' there may be multiple encounters with a host as different types of cues (visual, olfactory, gustatory, etc.) are encountered	Miller and Strickler 1984
Host finding	'to behave so as to establish and maintain proximity' with a plant perceived by the nervous system of the insect, does not imply directionality in motion	Miller and Strickler 1984
Host location	Host finding through movements with directionality	Miller and Strickler 1984
Host recognition	A match between the cues received and the acceptance criteria of the insect	Dethier 1982, Schoonhoven et al. 2005

Table 1.3. Types of stimuli affecting insect behavior before the insect is in physical contact with a plant (Dethier et al. 1960).

Term	Definition
Arrestant	A stimulus that causes insects to reduce their speed, either by slowing or ceasing movement or increasing their turning rate
Locomotor stimulant	A stimulus that causes insects to disperse
Attractant	A stimulus that 'causes insects to make oriented movement towards its source'
Repellent	A stimulus that 'causes insects to make oriented movement away from its source'

Table 1.4. Types of stimuli affecting insect feeding behavior when the insect is in contact with the plant (Beck 1965).

Term	Definition
Incitant	A stimulus that increases the probability that biting or piercing of a host plant will take place
Suppressant	A stimulus that decreases the probability that biting or piercing of a host plant will take place
Stimulant	A stimulus that increases the probability that feeding will continue once it has started
Deterrent	A stimulus that decreases the probability that feeding will continue once it has started

Table 1.5. Reported worldwide distribution of *Orthotomicus. erosus*.

Location	References
California	Lee et al. 2005, 2007, 2008
Azores	Bright and Skidmore 1997
Finland ^{2,3}	Bright and Skidmore 1997
Portugal	Bright and Skidmore 1997
Sweden ^{1,3}	Bright and Skidmore 1997
Algeria	Wood and Bright 1992
Egypt	Wood and Bright 1992
Libya	Wood and Bright 1992
Madeira Island	Wood and Bright 1992
Morocco	Wood and Bright 1992
South Africa ¹	Wood and Bright 1992
Swaziland ¹	Eglitis 2000
Tunisia	Wood and Bright 1992
China (Anhui, Fujian, Guangdong, Guangxi, Guizhou, Henan, Hunan, Jiangsu, Jiangxi, Liaoning, Shanxi, Shandong, Sichuan, Yunnan, and Zhejiang Provinces)	Wood and Bright 1992
Iran	Wood and Bright 1992
Israel	Wood and Bright 1992
Jordan	Wood and Bright 1992
Syria	Wood and Bright 1992
Turkey	Wood and Bright 1992
Tajikistan ¹	Eglitis 2000
Bulgaria	Wood and Bright 1992
Corsica	Wood and Bright 1992
United Kingdom ^{1,3}	Wood and Bright 1992, Eglitis 2000
France	Wood and Bright 1992
Greece	Wood and Bright 1992
Italy	Wood and Bright 1992
Poland	Wood and Bright 1992
Romania	Eglitis 2000
Sardinia	Wood and Bright 1992
Spain	Wood and Bright 1992
Switzerland	Wood and Bright 1992
former USSR	Wood and Bright 1992
former Yugoslavia	Wood and Bright 1992
Chile ¹	Wood and Bright 1992

¹ introduced, ² intercepted only

³ reported but established populations unlikely

Table 1.6 Reported hosts of *Orthotomicus erosus*

Hosts	References
<i>Abies pinsapo</i> ¹	Wood and Bright 1992
<i>Cedrus atlantica</i>	Bright and Skidmore 1997
<i>Cedrus libani</i> ¹	Wood and Bright 1992
<i>Cupressus arizonica</i>	Mendel and Halperin 1982
<i>Cupressus sempervirens</i>	Mendel and Halperin 1982
<i>Larix laricina</i> ²	Lee et al. 2008
<i>Picea glauca</i> ²	Lee et al. 2008
<i>Picea mariana</i> ²	Lee et al. 2008
<i>Picea orientalis</i> ¹	Wood and Bright 1992
<i>Pinus armandii</i>	Eglitis 2000
<i>Pinus banksiana</i> ²	Lee et al. 2007, Lee et al. 2008
<i>Pinus brutia</i> ³	Mendel and Halperin 1982, Bright and Skidmore 1997, Lee et al. 2007
<i>Pinus canariensis</i> ³	Mendel and Halperin 1982, Bright and Skidmore 1997, Lee et al. 2007
<i>Pinus caribaea</i>	Mendel and Halperin 1982
<i>Pinus contorta</i> ²	Lee et al. 2007
<i>Pinus coulteri</i>	Eglitis 2000
<i>Pinus echinata</i>	Mendel and Halperin 1982
<i>Pinus eldarica</i>	Mendel and Halperin 1982
<i>Pinus elliotii</i>	Lee et al. 2007
<i>Pinus halepensis</i> ³	Mendel and Halperin 1982, Wood and Bright 1992, Lee et al. 2007
<i>Pinus jeffreyi</i> ³	Lee et al. 2007, Lee et al. 2008
<i>Pinus kesiya</i>	Eglitis 2000
<i>Pinus lambertiana</i> ²	Lee et al. 2007, Lee et al. 2008
<i>Pinus maritimus</i>	Mendel and Halperin 1982, Wood and Bright 1992
<i>Pinus masoniana</i>	Eglitis 2000
<i>Pinus monophylla</i> ²	Lee et al. 2007, Lee et al. 2008
<i>Pinus mugo</i> subsp. <i>uncinata</i>	Eglitis 2000
<i>Pinus nigra</i>	Mendel and Halperin 1982, Bright and Skidmore 1997
<i>Pinus nigra austriaca</i>	Bright and Skidmore 1997
<i>Pinus nigra cevennensis</i>	Bright and Skidmore 1997
<i>Pinus nigra mauretanic</i>	Bright and Skidmore 1997
<i>Pinus nigra pallasiana</i>	Bright and Skidmore 1997
<i>Pinus patula</i>	Eglitis 2000
<i>Pinus pinaster</i>	Wood and Bright 1992
<i>Pinus pinea</i> ³	Mendel and Halperin 1982, Bright and Skidmore 1997, Lee et al. 2007
<i>Pinus ponderosa</i> ²	Lee et al. 2007, Lee et al. 2008

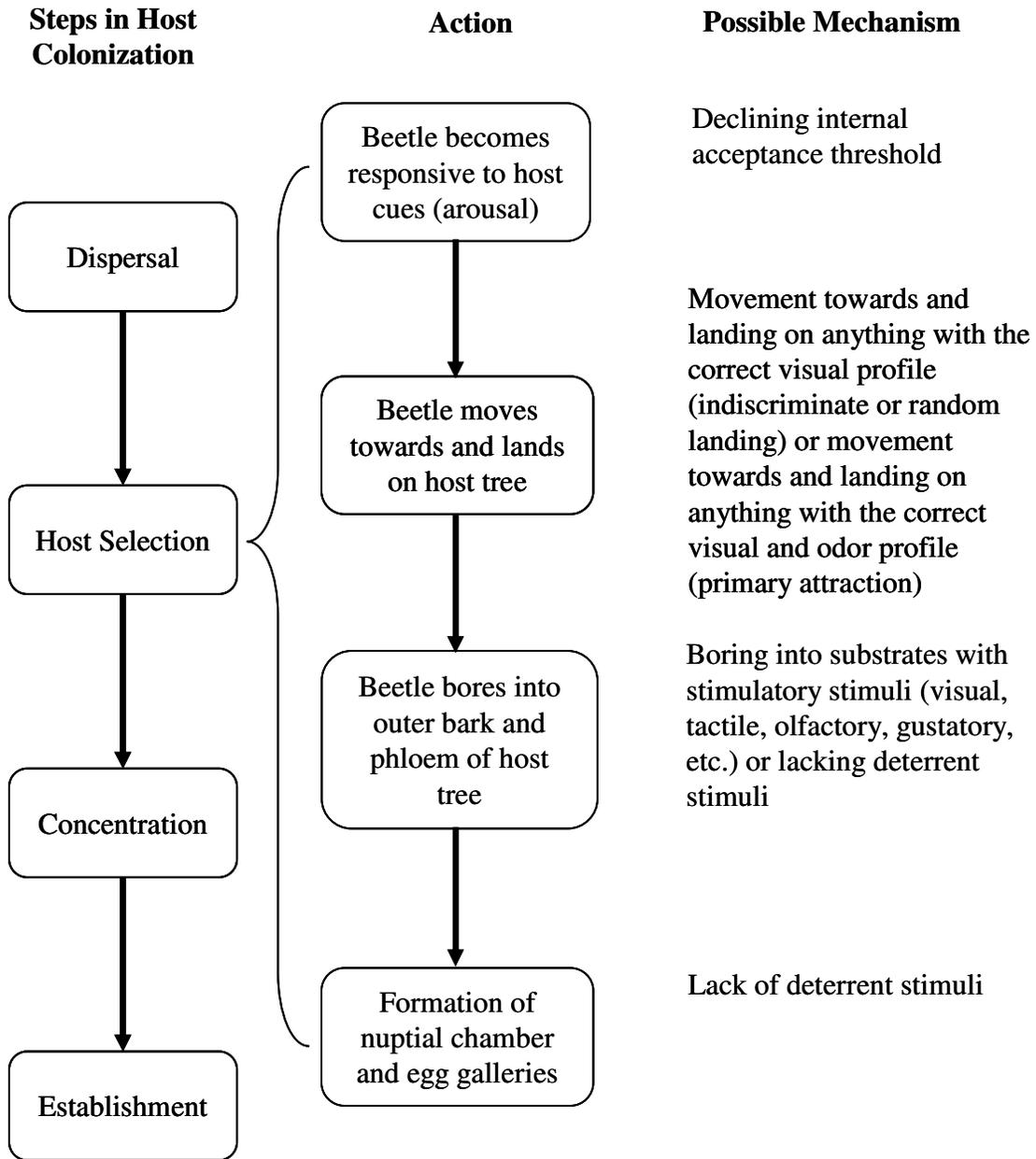
Hosts	References
<i>Pinus radiata</i> ³	Mendel and Halperin 1982, Bright and Skidmore 1997, Lee et al. 2007
<i>Pinus resinosa</i> ²	Lee et al. 2007, Lee et al. 2008
<i>Pinus sabiniana</i> ³	Lee et al. 2007, Lee et al. 2008
<i>Pinus strobus</i> ²	Lee et al. 2007, Lee et al. 2008
<i>Pinus sylvestris</i> ³	Bright and Skidmore 1997, Lee et al. 2007
<i>Pinus tabuliformis</i>	Eglitis 2000
<i>Pinus taeda</i> ²	Lee et al. 2007, Lee et al. 2008
<i>Pinus yunnanensis</i>	Eglitis 2000
<i>Pinus</i> spp.	Wood and Bright 1992
<i>Psuedotsuga menziesii</i>	Bright and Skidmore 1997

¹ rarely reported in this host

² recorded as a host in the laboratory, not observed as a host in the field

³ recorded as a host in the U.S.

Figure 1.1 Steps in bark beetle host colonization (*sensu* Wood 1982a). Beetles disperse from the brood log and select (accept one of several alternatives) a host tree. During host acceptance, beetles 1) become aroused to accept a host, possibly due to a declining internal acceptance threshold (Courtney et al. 1989), 2) approach and land on a tree via indiscriminate landing (Moeck et al. 1981) or primary attraction (Person 1931), 3) boring into the outer bark and phloem of the tree, possibly based on the presence of stimulants (Raffa and Berryman 1982) or lack of deterrents (Jermy 1966), and 4) form nuptial chambers and egg galleries, possible due to lack of deterrent compounds (Furniss 1976).



**Chapter 2. Acceptability and suitability of novel North American hosts for
Orthotomicus erosus, a potential invasive bark beetle**

ABSTRACT:

To predict whether an herbivorous pest insect will establish in a new area, the potential host plants must be known. For bark beetles, host plants must be selected by the adults and suitable for larval development. The preference-performance hypothesis predicts that adults will select only tree species suitable for their larvae. We tested five species of eastern North American conifers and one angiosperm for adult acceptance and larval suitability for the Mediterranean pine engraver, *Orthotomicus erosus* (Wollaston). Certain species, such as red pine, *Pinus resinosa* Aiton, and white spruce, *Picea glauca* (Moench) Voss, were entered by adult beetles and supported larval development to the extent of adult replacement. Others such as balsam fir, *Abies balsamea* (L.) Mill., eastern hemlock, *Tsuga canadensis* (L.) Carriere, and tamarack, *Larix laricina* (Du Roi) Koch stimulated entry but did not support development at or above the replacement rate. The presence of tree species acceptable to adults but unsuitable for development may affect the ability of *O. erosus* to establish across North America.

KEY WORDS: Mediterranean pine engraver, invasion biology, establishment, host range expansion, novel host association, Pinaceae, Scolytidae

INTRODUCTION:

Establishment success of an invading herbivorous insect depends in part on the ability of the newly arrived insect to select suitable host plants for feeding and oviposition. Host-selecting individuals in a new environment will encounter a novel set of host and non-host plants, and they must be pre-adapted to distinguish between them (Jermy 1988). Since host plants from the native range will probably not be present, high motivation to oviposit by the invading adult insects cause them to lower their threshold for host acceptance, leading to the use of plants that would not normally be attempted (Dethier 1982, Courtney and Kibota 1990).

For herbivorous insects whose progeny do not have a high dispersal capacity, the choice of larval host is determined by the ovipositing female (Janz 2002). Although adult host acceptance behavior is based on long-range and short-range cues, acceptance of the plant when the insect is in contact with it is a critical step. The preference-performance hypothesis predicts that females will select plants that maximize the fitness of their progeny (Wiklund 1975, Jermy 1984, Scheirs and De Bruyn 2002). However, oviposition on sub-optimal or unsuitable hosts may occur because the adults use cues to determine suitability of a novel plant that are not correlated with larval performance (Rodman and Chew 1980, Gripenberg et al. 2007). The phenomenon occurs during plant invasions; some herbivorous insects will oviposit on introduced plants even though those plants are unsuitable for their offspring (Straatman 1962, Chew 1977, Courtney 1981, Harris et al. 2001, Barre et al. 2002, Graves and Shapiro 2003, Badenes-Perez et al. 2006, Casagrande and Dacey 2007).

The Mediterranean pine engraver, *Orthotomicus erosus* (Wollaston), is a Eurasian bark beetle that has recently invaded California and colonizes several species in the genus *Pinus* (Lee et al. 2005). *Orthotomicus erosus*, one of the most frequently intercepted bark beetles in cargo and solid wood packing material entering North America (Brockerhoff et al. 2006b, Haack 2006), has successfully established in South Africa, Swaziland, The Republic of Tajikistan, and Chile (Eglitis 2000). The beetle is likely to spread to the rest of North America, either through new introductions or movement from California on its own or assisted by humans (Eglitis 2000). Potential impacts on native North American conifers are a concern (Lee et al. 2008).

If *O. erosus* does spread across North America, it will encounter genera and species of conifers with which it has no history of association. The complete host range of *O. erosus* and its potential for host shifting is unclear. Within its native and adventive ranges, *O. erosus* has been reported on many pines (*Pinus* spp.) (Mendel and Halperin 1982, Wood and Bright 1992, Arias et al. 2005), and it may also utilize Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), oriental spruce (*Picea orientalis* (L.)), Spanish fir (*Abies pinsapo* Boiss.), cedar (*Cedrus libani* A. Rich., *C. atlantica* (Endl.) Manetti ex Carriere) (Pinaceae) (Wood and Bright 1992, Bright and Skidmore 1997) and Mediterranean cypress (*Cupressus sempervirens* L.) (Cupressaceae) (Mendel and Halperin 1982). Recent host testing on 18 conifer species native to North America showed that larvae can develop in all pine (*Pinus*) species tested, as well as Douglas-fir (*Pseudotsuga menziesii*), and spruce (*Picea glauca* (Moench) Voss, *P. mariana* (Mill.) B.S.P.). A small amount of reproduction occurred on tamarack (*Larix laricina* (Du Roi) Koch) (Pinaceae), but not white fir (*Abies concolor* (Gond. & Glend.) Hildebr.) (Pinaceae),

incense cedar (*Calocedrus decurrens* (Torr.) Florin), or coast redwood (*Sequoia sempervirens* Lamb) (Cupressaceae) (Lee et al. 2008). In these host range studies, adult *O. erosus* were placed directly into drilled entrance holes into the phloem of the test species, but the extent to which *O. erosus* will initiate tunneling and gallery construction in various hosts is unknown and was further evaluated in our studies.

This paper reports host range testing on *O. erosus* under quarantine conditions. Our objective was to test several eastern North American conifers for acceptability to adults for boring and suitability for larval development. We test the hypothesis that when confronted with novel hosts, the adult beetles will enter the outer bark and bore in the phloem of those tree species that support the development of their larvae, but not those that do not support larval development. This paper reports that logs of species from at least two genera of Pinaceae in eastern North America are suitable for the development of *O. erosus*, but that beetle adults attack a wider range of tree species, some of which do not support larval development.

METHODS:

Beetle Colony: Cut logs of Aleppo pine, *Pinus halepensis* Mill., infested with *O. erosus* were collected in Fresno, CA in August 2006, July 2007, and August 2007. Bolts were held in emergence boxes (Browne 1972) in Davis, CA, and emerged adults were shipped to the MAES/MDA Containment Facility in St. Paul, MN (USDA APHIS Permit 7-4447). Adults were placed on logs of red pine (*Pinus resinosa* Aiton) collected from the University of Minnesota Research and Outreach Center (Grand Rapids, MN). Rearing bolts (approximately 50 cm x 15 - 40 cm diam.) were used within 10 wk after harvest. To minimize desiccation, cut surfaces of these bolts were sealed with paraffin wax (Candle Crafting Products Premium Candle Wax, Yaley Enterprises, Redding, CA). Between harvest and use in the colony, logs were maintained in a greenhouse at 7-13°C during the day and 4-10°C at night.

At least 20 adults were placed on the surface of each bolt, and these artificially infested bolts were placed in 38 cm diameter x 92 cm length cylinders of 0.75 cm thick cardboard, sealed at each end with ventilated end caps. During rearing, logs were maintained at 25°C, 16:8 L:D in a growth chamber. Under these conditions, beetle generations matured in approximately 6 wk. New colonies were initiated after one or two generations had developed in a red pine log. Under our rearing conditions and in the field *O. erosus* adults tend to remain in the brood log rather than emerge as long as the log remains moist enough (Mendel and Halperin 1982). In our colony, this led to severe competition and reduced populations of the beetle. Therefore, beetles used for rearing and experiments were obtained by peeling the bark and phloem of the colony logs and

manually extracting adult beetles. All handling procedures were approved by the USDA APHIS Plant Protection and Quarantine Division Permit Number 74447.

Collection of Host Material: Trees were felled at the University of Minnesota Research and Outreach Center (Grand Rapids, MN). Cut bolts (approximately 50 cm x 11 – 34.5 cm diam.) were sealed with paraffin wax and stored in a greenhouse in St. Paul, MN under the same conditions as logs used for colony maintenance until they were used in experiments. The species were: Red pine (*Pinus resinosa*), white spruce (*Picea glauca*), tamarack (*Larix laricina*), eastern hemlock (*Tsuga Canadensis* (L.) Carrière), balsam fir (*Abies balsamea*), and paper birch (*Betula papyrifera* Marsh.). Most species were collected in two years, but because of difficulty in locating specimens, hemlock collections were made only in 2007. Pine, spruce, larch, and fir were collected 13 September 2006; with an additional pine and birch collection on 22 October 2006. In 2007, a collection of two hemlock trees was made on 13 June, and two trees per species of pine, spruce, larch, hemlock, fir, and birch were collected 3 July. Two bolts from two individuals per test species were used for developmental suitability testing in 2006 and 2007 (8 bolts/species total). Bark, phloem, and xylem from two individuals per species (four from the hemlock collection) from the 2007 collection was used for the adult acceptance assay.

red pine was used as a positive control for all experiments because many pines are reported hosts of *O. erosus* (Wood and Bright 1992, Lee et al. 2008). As an angiosperm, paper birch was considered a highly unlikely host of *O. erosus*, and therefore used as a negative control.

Adult Acceptance Study: Adult acceptance of the potential hosts collected in July and August 2007 (pine, spruce, larch, hemlock, fir, and birch) was determined by a simultaneous no-choice experiment in a randomized complete block design. In a method similar to that of Elkinton and Wood (1980), bark discs (outer bark down to xylem) were cut with a 5 cm diam. hole saw and removed with a draw knife. Each disc was embedded in paraffin wax in its own 9 cm diam. plastic Petri dish. One beetle was placed on the bark disc for 72 h at 25°C, 16:8 L:D in a growth chamber. A few disc arenas for each tree species were ruined because the bark curled away from the phloem and wax overnight, and beetles were not placed in these arenas. The assay was run for 72 h to minimize false negative results (Tallamy 2000, Barton Browne and Withers 2002). In preliminary experiments we observed a large degree of beetle mortality after 72 h (data not presented). In addition, a 72 h assay time following readings at 15 min, 24 h and 48 h has been used in acceptance experiments with other species of bark beetles (Wallin and Raffa 2000).

At approximately 24 and 48 h, each dish was observed and the status of the beetle (living and visible, dead, boring, or not visible and not creating boring dust) was noted. At the end of the experiment, all the dishes were placed in a frost-free freezer (~ -20 °C) for at least 24 h. The discs were dissected and the position of the beetle, boring activity in the outer bark, and length of tunnel in the phloem layer were recorded.

The acceptance assay was conducted in six complete blocks between 17 July and 8 October 2007. In total, 30 male and 30 female beetles (5 beetles of each sex per block) were assayed on bark from each tree of each species and a control consisting of only wax. All beetles used in the acceptance study had been extracted from colony logs 2 d before

the start of the block and maintained in Petri dishes with moist filter paper in the growth chamber used for colony rearing. Bark disc arenas were constructed the day before the start of each block.

The effect of beetle sex, host species, block, and the two-way interactions on each stage of the acceptance sequence (Figure 2.1) were analyzed with logistic regression with a Wald χ^2 . Explanatory variables were added or removed from the model by using forward, backward, and stepwise selection (PROC LOGISTIC, SAS Institute Inc. 2004). In all cases, the selection methods yielded the same explanatory variables with $\alpha = 0.05$. When the effect was significant, contrasts among the treatments were conducted. The observed data for length bored in phloem by the beetles that bored into the outer bark and reached the phloem did not pass the Shapiro-Wilk test for normality ($W = 0.678$, $P < 0.0001$, PROC UNIVARIATE, SAS Institute Inc. 2004). A nonparametric Kruskal-Wallis test was used to test for differences in tunnel lengths between beetles that remained in their tunnels and those that abandoned them (PROC NPAR1WAY, SAS Institute Inc. 2004). Using the Kruskal-Wallis test with a Bonferroni-adjusted α of 0.0033 (PROC NPAR1WAY, SAS Institute Inc. 2004), comparisons of tunnel length were then made between tree species for beetles that abandoned their tunnels and those that remained.

Larval Suitability Study: The experiment followed a randomized incomplete block design because of space constraints in the quarantine facility. Block 1 was started on 27 October 2006 and included two bolts from each of three red pine trees (two trees from the Sept 2006 collection and one from October 2006), two white spruce, tamarack, and balsam fir trees, and one paper birch tree (20 bolts total). Block 2 was started on 21

August 2007 and included two bolts from each of four eastern hemlock trees (two trees collected June 2007, and two from July 2007) and two red pine, white spruce, tamarack, balsam fir, and paper birch trees (28 bolts total).

The methods for the developmental suitability study were similar to those of Lee et al. (2008). Using a power drill with a brad point bit, three holes (3.125 mm diameter) were drilled through the bark and phloem and just into the sapwood of the bolts. To ensure that at least one male per entrance hole would survive, two male beetles were placed into each hole and escape was prevented by taping a piece of metal screening over the hole. Two days later, three female beetles were added to each hole and the screening was replaced. Using this method, bark beetles will tunnel and oviposit in a wide variety of potential host plants (Furniss 1976, Lee et al. 2008). To ensure that beetles had adequate time to complete a generation on red pine, colony logs started at approximately the same time were peeled every few weeks. The experiment was ended when the beetle population in the colony logs consisted of mainly adults and very young larvae (indicating that the second generation was starting). All bolts were peeled. We counted all life stages of beetles present, with the exception of very small larvae in red pine (the second generation). All beetles that were counted (larvae, pupae, and adults) were assumed to represent a viable adult in the next generation, whether or not it had time to reach the adult stage during the experiment.

The number of viable offspring observed was tested for normality by using the Shapiro-Wilk test (PROC UNIVARIATE, SAS Institute Inc. 2004). The effect of species and block (spring or fall), variation among trees, and variation within a tree on the number of viable offspring observed per bolt was analyzed by using ANOVA followed

by Tukey's HSD with $\alpha = 0.05$ (PROC MIXED, SAS Institute Inc. 2004). The proportion of beetles in various life stages (adult, teneral, pupa, large larva, or small larva) recovered from the logs was analyzed by ordinal logistic regression with species, collection season, and their interaction as explanatory variables (PROC LOGISTIC, SAS Institute Inc. 2004). Variables were selected with forward, backward, and stepwise selection options with $\alpha = 0.05$. All three selection methods for the regression model yielded the same result. In a separate analysis, the 95% confidence interval was estimated for the mean number of viable offspring produced in each species (PROC TTEST, SAS Institute Inc. 2004) to determine whether the mean was less than, equal to, or greater than 15 viable offspring per log (the replacement rate).

RESULTS:

Adult Acceptance Study: About one-tenth of the beetles that were used in the study died during the first 2 d of the assay without any boring activity and were excluded from further analysis. The probability of remaining alive did not vary by sex, host species, or block ($N_1 \rightarrow N_2$ in Table 2.1, $df = 6$, $\chi^2_w = 2.67$ $P = 0.85$).

We viewed host acceptance by *O. erosus* as a stepwise series of behaviors (Figure 2.1), and we analyzed the host acceptance behaviors independently of each other. However, the numbers of beetles analyzed for a certain step in the host acceptance sequence was determined by the number of beetles that completed the previous steps (i.e., the number analyzed at N_2 for a given species depends on the number of beetles that completed N_1) (Table 2.1). Thus, as host acceptance progressed, the design became more and more unbalanced, and the number of beetles completing each step declined with step (Table 2.1).

There were significant differences among tree species in the probability that beetles would initiate boring into the outer bark ($N_2 \rightarrow N_3$ in Table 2.1, $df = 6$, $\chi^2_w = 28.25$, $P < 0.0001$, Figure 2.2). Beetles had the highest probability of boring into red pine, white spruce, and balsam fir. White spruce, tamarack, eastern hemlock, and balsam fir also formed an intermediate group; the lowest probability of boring was associated with paper birch. The proportion of the beetles that bored into the outer bark that subsequently reached the phloem did not differ among species ($N_3 \rightarrow N_5$ in Table 2.1, $df = 5$, $\chi^2_w = 8.76$, $P = 0.12$). There were no differences among tree species in the proportion of beetles that abandoned their tunnels after reaching the phloem ($N_5 \rightarrow N_6$ in

Table 2.1, $df = 5$, $\chi^2_w = 9.04$, $P = 0.11$). Beetle sex or experimental block did not significantly affect any of these measures.

When the tree species were separated into developmentally suitable hosts (red pine and white spruce) and other hosts (all others) based on the results of the developmental suitability study (see below), the male and female beetles had a higher probability of boring into suitable species versus unsuitable species ($N_2 \rightarrow N_3$ in Table 2.1, $df = 1$, $\chi^2_w = 9.08$, $P = 0.0026$). This result is significant even though pairwise comparisons of some individual unsuitable tree species were statistically indistinguishable from the suitable species for this step.

Once beetles had bored into the outer bark and reached the phloem, the length of boring in the phloem was different depending on whether the beetles subsequently abandoned the disc ($df = 1$, $\chi^2_{kw} = 7.6009$, $P = 0.0058$). Beetles abandoning the disc constructed shorter tunnels than those that did not abandon. For the beetles that remained in their disc, the length of boring differed between red pine and eastern hemlock ($df = 1$, $\chi^2_{kw} = 17.6787$, $P < 0.0001$), red pine and balsam fir ($df = 1$, $\chi^2_{kw} = 18.5654$, $P < 0.0001$), white spruce and eastern hemlock ($df = 1$, $\chi^2_{kw} = 9.3448$, $P = 0.0022$), and white spruce and balsam fir ($df = 1$, $\chi^2_{kw} = 12.5248$, $P = 0.0004$) (Figure 2.3). The length bored by beetles in birch was not different from that in any of the conifer species, but this value is probably due to the small number of beetles that bored in birch ($N_6 = 6$), and the influence of one beetle in particular that constructed a very long tunnel. Few beetles that reached the phloem abandoned their disc (12 out of 155). For the beetles that reached the phloem and abandoned their disc, there were no significant differences in the length of boring in the phloem among the test tree species.

Larval Suitability Study: In bolts of various tree species, beetles would often tunnel in the phloem without constructing an obvious egg gallery; in some tree species, beetles laid eggs in winding galleries that were very different in shape from the egg galleries constructed in red pine. *Orthotomicus erosus* is known to form different gallery patterns on different hosts (Eglitis 2000). Because of this gallery variation, we were unable to count the number of egg galleries contained in each bolt.

Tree species affected the total number of offspring produced per bolt ($df = 6$, $F = 5.16$, $P = 0.0005$) (Figure 2.4), but there was no effect of block ($df = 1$, $F = 0.86$, $P = 0.3596$). The most beetles were produced in red pine. The number of viable offspring observed in individual bolts was greater than or equal to the number of parent beetles introduced (the replacement rate) only in red pine (95% CI 7.64 - 52.76 viable offspring) and white spruce (95% CI 2.25 - 17.50 viable offspring). The single tamarack bolt where viable offspring were observed contained 29% adults, 14% pupae, and 57% larvae. White spruce bolts with viable offspring contained 24% adults, 9% pupae, and 67% larvae; red pine bolts with viable offspring contained 47% adults, 6% pupae, and 47% larvae. This variation in the life stage of the beetles did not vary by tree species, block, or their interaction according to ordinal logistic regression.

DISCUSSION:

We expected that adult *O. erosus* would accept all or a subset of novel host species that were suitable for larval development and reject unsuitable species. However, we found that although the adults accepted the species that were suitable for larval development (red pine and white spruce), they also accepted several species that were not suitable (balsam fir and to some extent tamarack and eastern hemlock). Thus, when faced with novel host plants during an invasion, adult *O. erosus* may oviposit in trees that are unsuitable for larval development.

The results of our suitability testing are very similar to those obtained by Lee et al. (2008). These results support field observations that *O. erosus* uses species of both pine (*Pinus*) and spruce (*Picea*) as hosts (Wood and Bright 1992, reviewed in Lee et al. 2008). *Orthotomicus erosus* has been collected on one species of fir (*Abies pinsapo*) (Wood and Bright 1992); other fir species were not suitable in laboratory experiments (Lee et al. 2008, this study). In Europe, native bark beetles are frequently able to utilize introduced tree species congeneric with their native hosts (Sauvard 2004).

Bark beetles appear to select hosts after they have sampled the outer bark or the phloem (Elkinton and Wood 1980, Hynum and Berryman 1980, Raffa and Berryman 1982, Pureswaran and Borden 2003). Some bark beetle species, such as *Ips paraconfusus* Lanier, are unable to distinguish between the outer bark of hosts and certain non-hosts (Elkinton and Wood 1980), and make their host determination only after sampling the phloem (Wood 1963, Elkinton and Wood 1980, Raffa et al. 1993). By analyzing differences in each step of host selection (Figure 2.1), I am able to show that, for the tree

species we tested, differences in host acceptance by *O. erosus* are driven by differences in the proportion of beetles boring into the outer bark. The stimulant or deterrent signals used in host selection by *O. erosus* can therefore be expected to occur in the outer bark (see Chapter 4). Understanding the nature of these signals could allow prediction of the acceptability of a novel tree species to *O. erosus*.

An interesting result of the host acceptability experiment was that male and female beetles had the same probability of host acceptance on the species tested. In descriptions of the life history of *O. erosus* (Mendel and Halperin 1982) and other members of Ipini (Wood 1982b), males are generally considered the host-finding sex, and females are reported to move to a host after males have excavated a nuptial chamber in the phloem. However, 54 - 97% of female *O. erosus* are already mated after overwintering or maturation feeding (Mendel 1983). Females could attempt to enter a host and reproduce whether males were present in the host or not, although this behavior is not yet documented in the field for *O. erosus*.

Similar female gallery initiation behavior has been reported under experimental conditions in other bark beetle species where males are reportedly the host-finding sex. For instance, female *Ips paraconfusus* initiated their own galleries 10% of the time when caged on logs, even though the logs were not crowded and males had already established galleries and were emitting pheromones (Wood 1963). In a field experiment, 4 out of 20 *I. paraconfusus* in galleries in a baited log were lone females (Elkinton and Wood 1980). Since aggregation is not necessary for beetles colonizing down wood, and crowding can incur a serious fitness cost in these situations (Byers 1989, Zhang et al. 1992), it may be advantageous for mated females to establish galleries on their own without advertising

the presence of the resource or their offspring to competitors and natural enemies. If this behavior occurs in the field, it could also increase the probability of establishment of an invading beetle population since mate finding after leaving the brood log would be unnecessary.

Presenting bark beetles with intact or ground discs of bark, phloem, or both is a well-established method for testing host acceptance (Wood 1963, Elkinton and Wood 1980, Raffa 1988, Klepzig et al. 1996, Wallin and Raffa 2000, 2004). Differences between tree species in both entry to the outer bark and length of tunneling have been observed in disc assays (Wallin et al. 2002). Laboratory host range testing often yields a broader host range than insects exhibit in the field (Zwolfer and Harris 1971, Pinski et al. 2005), and the bark and phloem disc assay method typically results in higher levels of host acceptance than are observed for standing live trees (Raffa 1988). However like most members of the Ipini, *O. erosus* is a secondary pest at low population levels, feeding on freshly killed or dying trees and only switching to live trees when beetle populations are very high (Arias et al. 2005). Thus, the presence of undefended fallen wood, as represented by our discs, not the presence of live and healthy trees, will determine the possibility of establishment by a small founding population of *O. erosus* in the field, making a conservative acceptance test such as a disc assay appropriate.

The results presented here do not address the searching behavior of *O. erosus* before the beetles are in contact with the bark surface. Although an acceptable and suitable host that is unattractive to the beetles at long range might be less utilizable than we predict, high attractiveness will not increase the reproductive output of bark beetles on a host that is unacceptable or unsuitable. Many bark beetles have rather indiscriminate

landing behavior (Elkinton and Wood 1980, Hynum and Berryman 1980, Moeck et al. 1981, Schroeder 1992), possibly because the volatile profiles of many conifers are qualitatively very similar (Pureswaran et al. 2004) or host acceptability decisions incorporate prior landing encounters (Wallin and Raffa 2002). Bark beetles have been reported to respond to volatile (Schroeder 1992, Byers et al. 2000, Huber and Borden 2000, Zhang et al. 2002, Pureswaran and Borden 2005, reviewed in Seybold et al. 2006) and non-odor (Campbell and Borden 2006) host cues at long range. However, a searching beetle that does not encounter attractive cues will eventually have to land due to exhaustion or because environmental conditions become unsuitable for flight. After landing, if a beetle contacts woody material it will encounter short range cues that may lead to acceptance of a non-attractive host.

Adult acceptance of unsuitable plants is known from systems involving invasive plants and native insects (Harris et al. 2001, Barre et al. 2002, Graves and Shapiro 2003, Badenes-Perez et al. 2006, Casagrande and Dacey 2007). However, the effect of this behavior on the success of an invading insect has seldom been considered. If accepted but unsuitable plants were prevalent enough, or preference for these species was strong, accepted unsuitable plants could act as a reproductive sink. My results suggest that *O. erosus* will accept an unsuitable host, which is a reproductive dead end. Bark beetle aggregation behavior might enhance the reproductive sink effect, if aggregation was induced on accepted unsuitable plants. Aggregation behavior could reduce the effect if aggregating beetles bypassed unsuitable species that they would normally accept to join an aggregation elsewhere. However, the low population levels expected for a founding population of an invasive species are unlikely to be large enough for successful

aggregation on a live host. Further work is needed to determine whether the presence of acceptable, unsuitable plants reduces the fitness of the beetle and affects the ability of the insect to establish and spread.

Most species introduced to a new habitat do not establish (Williamson 1996, Mack et al. 2000). Others establish in only some of the areas where they are introduced or have much larger effects in some areas than others (Houston and Valentine 1977, Williamson 1999, Tobin and Whitmire 2005). These experiments show that adults of invading *O. erosus* populations are likely to accept some tree species that are not suitable for the development of their larvae. Laboratory choice experiments and field work will be needed to assess whether unsuitable hosts can act as reproductive sinks. The presence of biotic or abiotic conditions in the new environment that prevent the invader from using resources in the new environment efficiently, such as accepted but unsuitable plants, may contribute to establishment failure. The presence of non-host species that affect insect behavior may help to explain the difficulty in predicting invasion success by potential pest species or biological control organisms (Williamson 1999, Mack et al. 2000, Worner 2002).

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Table 2.1. Total number of beetles arriving at stages of the host acceptance sequence for each potential host species. N_x labels refer to Figure 1.

	N_1	N_2	N_3	N_4	N_5	N_6	N_7
Red Pine	119	97	49	15	34	30	3
White Spruce	118	100	38	6	32	31	0
Tamarack	121	100	26	10	16	15	1
Eastern Hemlock	239	201	74	22	52	43	7
Balsam Fir	119	101	37	16	21	18	3
Paper Birch	110	95	12	6	6	6	0

Figure 2.1. Flow chart showing the possible outcomes involving a bark beetle during the host acceptance bioassay.

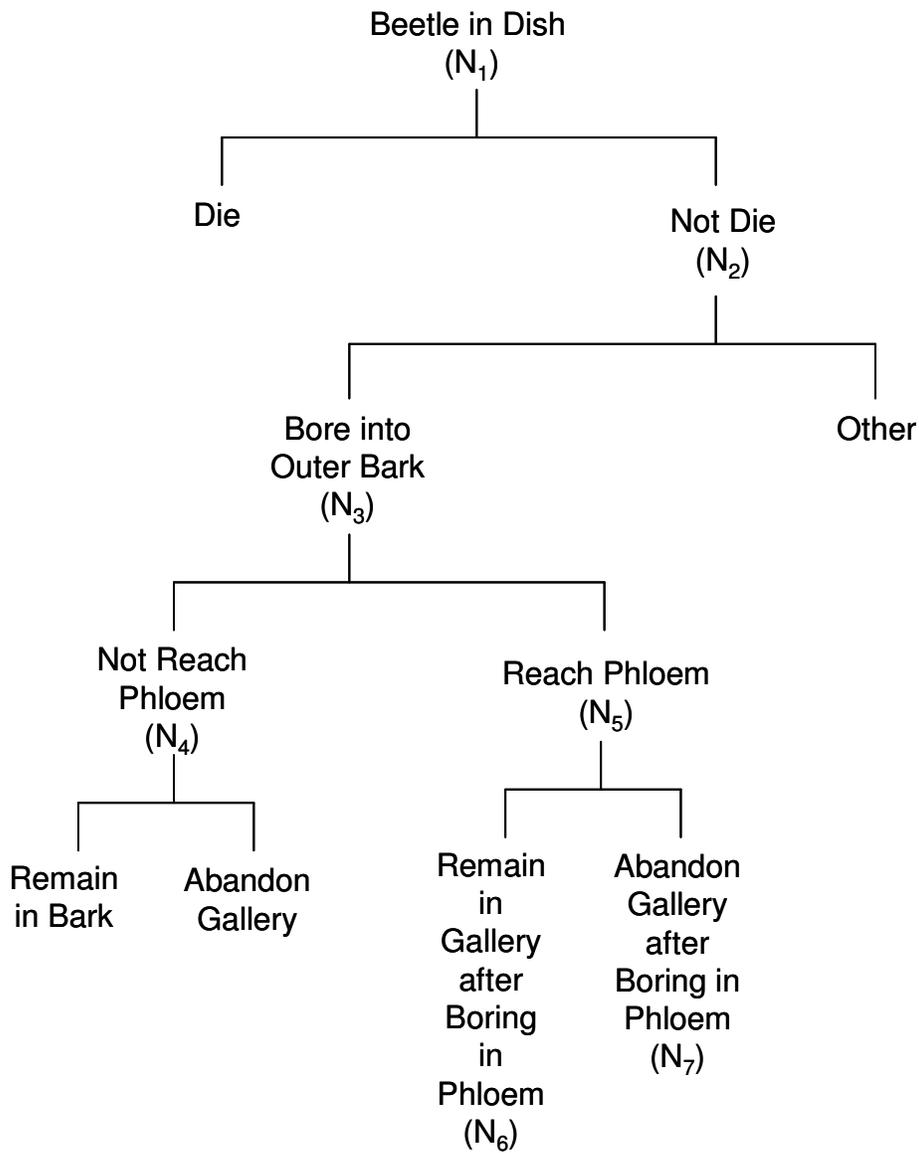


Figure 2.2. Probability (\pm binomial standard error) of boring into the outer bark of bark discs from potential host species by *Orthotomicus erosus*. Bars with the same letter are not significantly different in a protected mean separation procedure in logistic analysis.

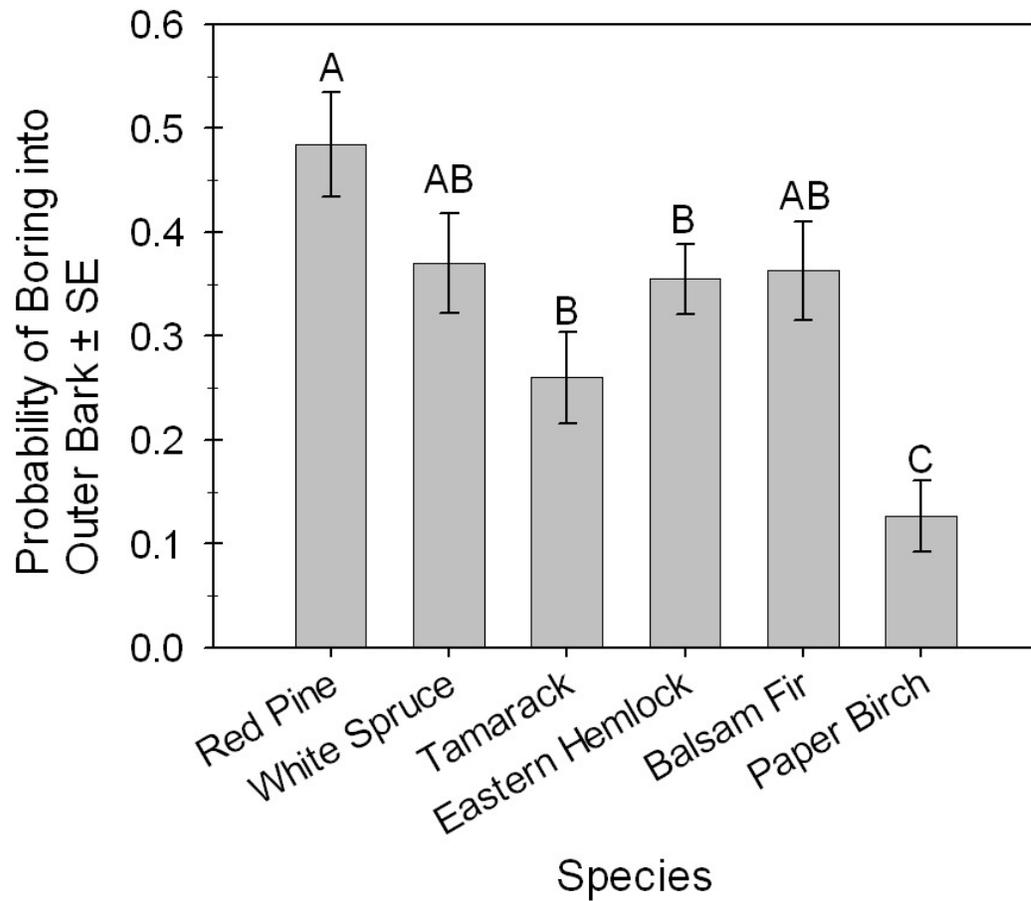


Figure 2.3. Length (mean \pm standard error) bored in the phloem of bark discs from potential host species by adult *Orthotomicus erosus* that bored into the outer bark of each species, reached the phloem, and did not subsequently abandon their galleries. Bars with the same letter are not significantly different according to multiple nonparametric Kruskal-Wallis comparisons. For the entire test, $\alpha = 0.05$; the Bonferroni adjustment was used to obtain the pairwise α level of 0.0033.

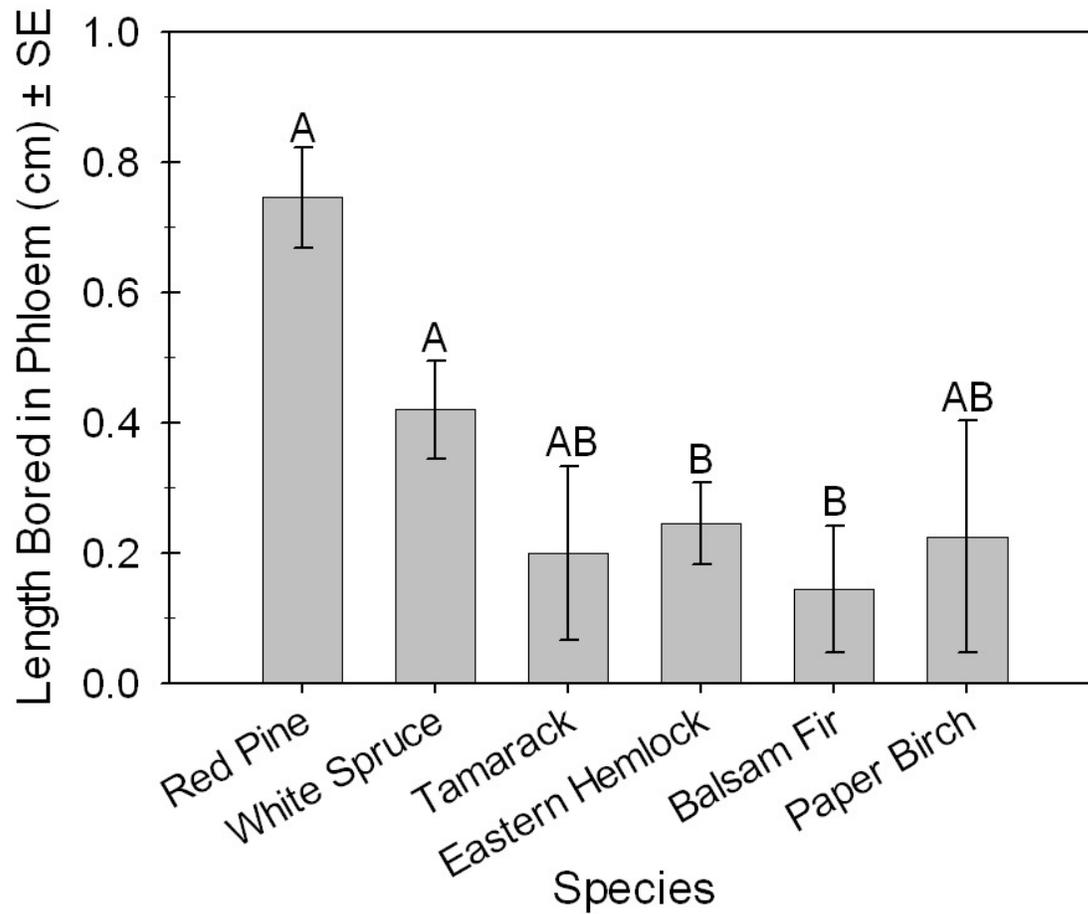
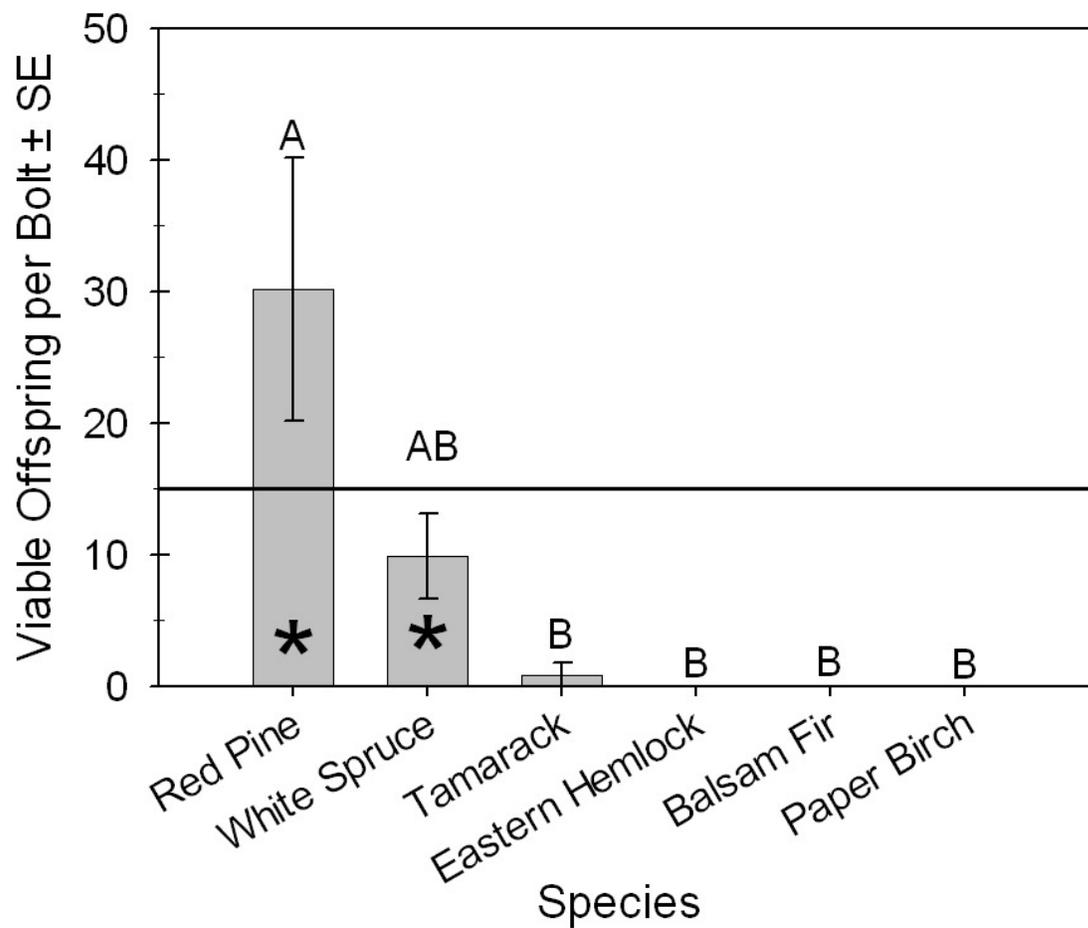


Figure 2.4. Total viable offspring produced (mean \pm standard error) in bolts of each potential host species by six male and nine female parents of *Orthotomicus erosus*. Bars with the same letter are not significantly different according Tukey's HSD with $\alpha = 0.05$. The horizontal line represents the number of offspring required to replace the parent beetles introduced to the log (replacement rate). * indicates host tree species where the 95% confidence intervals for viable offspring produced overlap the replacement rate.



Chapter 3. Walking Response of the Mediterranean Pine Engraver, *Orthotomicus erosus*, to Host and Non-host Volatiles in a Laboratory Olfactometer

Abstract When an herbivorous insect enters a new geographic area, it will select host plants based on short and long distance cues. A bark beetle that has been recently introduced to North America, the Mediterranean pine engraver, *Orthotomicus erosus* (Wollaston), has a potentially wide host range. However, its actual North American host range is currently unknown. *Orthotomicus erosus* can develop in some species of North American trees, but the beetles will bore through the outer bark of a larger set of tree species if they come in contact with the bark. The long-distance response of the beetles to tree odors may provide an additional mechanism of host recognition and selection, particularly during the earliest phases of host colonization. A laboratory olfactometer was used to study the response of *O. erosus* to odor cues from the bark and phloem of several North American tree species. The beetle moved toward odors of paper birch, *Betula papyrifera* Marsh., more than would be expected by chance, but had a neutral response to odors of red pine, *Pinus resinosa* Aiton, white spruce, *Picea glauca* (Moench) Voss, tamarack, *Larix laricina* (Du Roi) Koch, eastern hemlock, *Tsuga canadensis* (L.) Carriere, and balsam fir, *Abies balsamea* (L.) Mill. Movement toward balsam fir occurred less often than movement toward red pine, eastern hemlock, and paper birch. The set of attractive tree species in the olfactometer differs from the set of species acceptable on contact and the set of species that support larval development. When confronted with novel non-host tree species, *O. erosus* may be attracted to trees that are not suitable for the development of its larvae.

Keywords Bark beetle • host range testing • Pinaceae • Scolytidae • sequential no-choice olfactometer

Introduction

Many species of herbivorous insects are capable of feeding and developing on several plant species, but only a subset of those plant species are encountered by adults and selected for oviposition. Plant species that support the development of the insect and are utilized in the field comprise the ecological host range of the insect (Schaffner 2001).

When the geographic range of an herbivorous insect expands during a biological invasion, the ecological host range may also expand if the herbivore is pre-adapted to select novel plant species that support offspring development (Jermy 1988).

However, new plant-herbivore associations can also lead to “host confusion” (Larsson and Ekbom 1995, Stastny et al. 2006), when the insect fails to distinguish plants that are suitable for reproduction from those that are unsuitable.

If a non-host plant has been frequently encountered by successive generations of an insect species, selection should act against those insects that select the non-host. However, if a plant species has not been associated with an insect species, as is the case for allopatric species, optimal host selection may not occur when the two species come together initially (Larsson and Ekbom 1995). Specialist insects, such as coniferophagous bark beetles, are capable of detecting a wide range of host and non-host volatiles (Shepherd et al. 2007), but these volatiles occur in complex odor mixtures, with very few compounds unique to single species (Chararas et al. 1982, Pureswaran et al. 2004, Shepherd et al. 2007). An invasive insect may misinterpret the information it receives from allopatric plants and deposit its eggs on a non-host, even when suitable hosts are present in the environment.

The Mediterranean pine engraver, *Orthotomicus erosus* (Wollaston) (Coleoptera: Scolytidae, sensu Wood 2007), is a bark beetle that was recently introduced to North America, with an established population in California (Lee et al. 2005, 2007). The beetle feeds on a variety of pines (*Pinus* spp), and has been reported in association with Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), oriental spruce (*Picea orientalis* (L.) Peterm.), Spanish fir (*Abies pinsapo* Boiss.), Lebanon and atlas cedar (*Cedrus libani* Rich., *C. atlantica* (Endl.) Manetti ex Carriere) (Pinaceae) (Wood and Bright 1992, Bright and Skidmore 1997, Lee et al. 2008) and Italian cypress (*Cupressus sempervirens* L.) (Cupressaceae) (Mendel and Halperin 1982). Within its native range, *O. erosus* seems to select hosts indiscriminately among four pine species, two spruces, and two firs, although all those plants were preferred to a sympatric cypress (Chararas et al. 1982). The diversity of reported hosts has led to concern that the beetle may attack of a wide range of North American trees should its current geographic range expand.

Host utilization behavior by bark beetles can be divided into four sequential steps: dispersal, host selection, concentration, and establishment (Wood 1982a). Dispersal and host selection are likely to be most important during the initial phases of a biological invasion. From the moment a mature beetle leaves its natal host, the beetle receives information from its environment and engages in a series of behaviors that ultimately lead to attempted reproduction. For various bark beetle species, there has been debate about the nature and importance of various environmental cues that bark beetles might use to accept hosts, and the point at which the final host selection decision is made. Non-host volatiles are known to deter some bark beetle species from landing on unsuitable hosts (Schroeder 1992, Schlyter et al. 2000, Huber and Borden 2001, Jactel et al. 2001,

Zhang and Schlyter 2004, Zhang et al. 2007b, Fettig et al. 2009). Host volatiles in the absence of any bark beetle semiochemicals are known to be attractive to some bark beetle species (Schroeder 1987, Brattli et al. 1998, Kohnle 2004, Erbilgin et al. 2007), but others appear to land indiscriminately on objects that have the correct visual profile (Hynum and Berryman 1980, Moeck et al. 1981). Certain bark beetle species are known to discriminate between host and non-host tree species when boring into the outer bark (Elkinton and Wood 1980, Wallin and Raffa 2002b) or after reaching the phloem (Wood 1963, Elkinton and Wood 1980, Raffa et al. 1993). Several conceptual models to explain host selection by bark beetles have been advanced (Graves et al. 2008).

Previous work suggests that *O. erosus* may not reliably distinguish hosts from non-hosts when adults are in contact with the outer bark of several species of uninfested North American trees. Although the beetle is able to feed, develop, and reproduce sufficiently to replace parents on North American species, including several pines (*Pinus* sp.), Douglas-fir, *Ps. menziesii*, and at least two spruces, *Picea glauca* (Moench) Voss and *Pic. mariana* (Mill.) Britton, Sterns, and Poggenb. (Pinaceae) (Lee et al. 2008, Walter et al. in review-a), adults bored into the outer bark of several unsuitable tree species including balsam fir, *Abies balsamea* (L.) Mill., and, to some extent, tamarack, *Larix laricina* (Du Roi) Koch, and eastern hemlock, *Tsuga canadensis* (L.) Carriere (all Pinaceae) (Walter et al. in review-a). However, the behavior of dispersing beetles may enable *O. erosus* to avoid contacting acceptable non-host trees. Although certain volatiles from non-host plants are known to interfere with the pheromone response of *O. erosus* (Zhang and Schlyter 2004), the beetles' use of plant volatiles in host selection has not been characterized.

To better understand the effects of odors from allopatric tree species on the behavior of *O. erosus*, this paper examines the walking response the response of adult *O. erosus* to the bark and phloem odors of six eastern North American tree species. Because of the limited North American geographic range of the beetle, these studies utilized an olfactometer in a biosecurity level 2 quarantine facility. These studies address two hypotheses 1) Odors from selected tree species elicit a behavioral response from *O. erosus*, and 2) Odors from trees that differ in their suitability or short-range acceptability also differ in their attractiveness to *O. erosus*.

Materials and Methods

The experiments took place in the Minnesota Agricultural Experiment Station/Minnesota Department of Agriculture Biological Level 2 Containment Facility in St. Paul, MN, USA. Beetles from California and from a colony maintained in St. Paul, MN were used for the experiments. All handling procedures were approved by USDA APHIS Plant Protection and Quarantine Division (Permit 74447).

Field Collection of Beetles

Cut logs of Italian stone pine, *Pinus pinea* L., and Aleppo pine, *Pinus halepensis* Mill., infested with *O. erosus* were collected during the summer of 2008 in Fresno County, CA, USA and held in emergence boxes (Browne 1972). Adults were allowed to emerge naturally and were kept in refrigerators (approx. 10°C) in jars with moist paper towel until shipment. On 24 June, 5 August, and 13 August 2008, emerged beetles were shipped to St. Paul, MN in insulated styrofoam boxes containing ice packs. All beetles were held in the dark at approximately 10°C and deprived of food until they were used in the experiment 1-20 d later.

Beetle Colony

A colony of *O. erosus* was maintained on cut logs of red pine, *Pinus resinosa* Aiton, (approximately 50 cm x 15 - 40 cm diam.), obtained from the University of Minnesota Northern Research and Outreach Center (UMN-NROC, Grand Rapids, MN). Before use in the colony, cut logs were stored in a greenhouse at heated 7-13°C during the day and 4-10°C at night if temperatures fell below these values. Cut surfaces of the cut logs were sealed with paraffin wax (Candle Crafting Products Premium Candle Wax, Yaley Enterprises, Redding, CA) within 2 d post-harvest to minimize desiccation, and the cut logs were used within 10 wk post-harvest.

The original source of this colony was collected in 2006 and 2007 from infested *P. halepensis* and in 2008 from infested *P. halepensis* and *P. pinea* in California as described above, and beetles were shipped in August 2006, July and August 2007, and June and August 2008. In the colony, the beetles were reared by placing at least 20 adults onto a cut log. The cut logs were held in cardboard tubes (92 cm x 38 cm diameter cylinders with a wall thickness of 0.75 cm), sealed at each end with ventilated end caps and placed in a growth chamber maintained at 25°C, 16:8 L:D. Under these conditions, one generation developed in approximately 6 wk and beetles were capable of re-infesting the same log.

New colonies were initiated after one or two generations in a log. Beetles used for the experiments or to maintain the colony were obtained by peeling the bark and phloem of the colony logs and manually extracting adults. Between removal from the brood log and use in the experiment, beetles were kept in plastic Petri dishes at approximately 10°C for at least 24 hr.

Field Collection of Cut Logs

Species of cut logs for host olfactory stimuli included: red pine, white spruce, tamarack, eastern hemlock, balsam fir and paper birch, *Betula papyrifera* Marsh. Two trees of each test species (14.5-27.5 cm diameter) were felled at UMN-NROC between 16 and 19 June 2008 and immediately cut to approximately 50 cm lengths. The cut logs were sealed with paraffin wax and stored for less than 8 wk under the same conditions as logs used for the colony.

Laboratory Olfactometer Design

Air was pushed by a pump through a filter (Big Hydrocarbon Trap, BHT-2, Agilent, Santa Clara, CA), then humidified in a 250 ml Erlenmeyer flask filled with distilled deionized water with the air inlet below the water level (Fig. 3.1). After being humidified, the airstream was split, with each side passing through a 150mm flowmeter (Size #3 Riteflow Flowmeter, Bel-Art Products, Pequannock, NJ, USA) and then into one of two 25 cm tall x 15 cm diameter glass volatile collection chamber with one-port lids (ARS, Inc., Gainesville, FL, USA). Each volatile chamber contained a plastic stand to ensure air flow around the stimulus. After passing through the volatile chamber, each airstream was split again, and passed into four walking chambers, which were each composed of a 22-cm-long piece of Nalgene Premium Tubing (0.7 cm ID, Fisher Scientific, Pittsburgh, PA, USA). All connections prior to the walking chambers were

made by using polytetrafluoroethylene (PTFE) tubing (Sigma-Aldrich, St. Louis, MO). (Assays were run concurrently in each of the four walking chambers, two for the clean control airstream and two for the odor-laden treatment airstream, to increase the throughput of the assay.)

A 15-cm course was marked on each walking chamber beginning 1 cm from the open end of the tube. The walking chamber was straightened by fastening it to a plastic test tube rack with wire twist ties. The four walking chambers were placed in a 56 x 60 x 60 cm experimental arena made of laminated wood (veneered particle board) with a white floor and white walls on three sides. The arena was illuminated by a fluorescent light fixture (two 40 W tubes, 60 cm long) placed 60 cm above the floor of the arena as described in Wyckhuys and Heimpel (2007) (Fig. 3.1). The airflow at both flowmeters was set to 400 ml/min and split downstream of the flowmeters, so that the airflow through the four individual walking chambers was 200 ml/min.

To initiate each 5 min behavioral assay, beetles were placed in the open end of the walking chamber with soft forceps. Beetle movements were monitored during the control and treatment exposures. The time when they passed the first mark of the 15 cm course, and the total distance that they moved in 5 min were recorded. If beetles turned and walked in the direction of the airflow and exited the chamber, the time that they left the chamber was recorded and the distance moved was considered -1.1 cm. If a beetle walked the entire 15 cm course, then the time when the beetle passed the end of the course was recorded and the distance was considered 15 cm. Each beetle was exposed to two airstreams, first in a control airstream, then in a treatment airstream. The same walking chamber was used for both exposures.

The stimulus chambers containing the controls and treatments were randomly assigned, as were the position of the control and treatment tubes within the white arena. Chambers and positions were reassigned before starting a new treatment. Each beetle was used only once in the experiment. Each walking chamber was used for one beetle before being cleaned; the stimulus chambers that contained bark and all downstream tubing were cleaned after each group of 20 beetles. The cleaning procedure involved washing the olfactometer components in soapy water, rinsing with hot water, and then rinsing again with 95% ethanol. Safety concerns precluded the use of other solvents within the quarantine facility. All parts of the olfactometer were thoroughly air dried before being reused.

Clean Air Assay

This study was designed to evaluate the tendency of *O. erosus* to move a longer or shorter distance the second time that they were placed in the olfactometer. In this trial, termed the clean air assay, a total of 60 males and 60 females were exposed to airstreams without volatiles in the control and treatment. Four blocks of 10 male and 10 female beetles were run concurrently with blocks three through six of the host attraction assay with field-collected or laboratory-reared beetles according to the source of beetles for the rest of the block. An additional 20 male and 20 female beetles were run between blocks two and three of the experiment and were from the laboratory colony.

Host Attraction Assays

This experiment involved 1440 beetles. The treatment stimulus was 200 g of freshly peeled bark and phloem from one of the six test tree species (2 trees per species were used in the experiment). Six blocks of the experiment were run, each consisting of 10 male and 10 female beetles per test tree. Each block contained one sample from each of the 12 test trees (2 trees / tested species). Three blocks consisted of field-collected beetles, and three consisted of laboratory-reared beetles.

Beetles moved an identical distance in the control and treatment airstreams in 257 cases; 189 of these were instances where a beetle moved out the back of the tube during both exposures. The cases where the beetles moved an identical distance were resolved by examining the time that the beetles took to move, with the higher velocity treatment being considered equivalent to moving a greater distance.

Sample Size

We used a power analysis to determine the total number of beetles needed to detect a 10% difference in the probability of moving further in the treatment airstream than in the control with 99% confidence (Ott and Longnecker 2001). This difference was chosen arbitrarily based on the results that we observed from the first two blocks, on our estimation of the magnitude of difference from random movement that would be biologically meaningful, and on a pragmatic estimate of how many beetles would be available for use in the experiment.

Data Analysis

The response variable for data analysis was the probability of an individual moving a greater upstream (i.e., against the direction of airflow) distance in the treatment airstream than in the control airstream. The control airstream was always presented first to avoid any effect that moving out of an odor-laden airstream might have on beetle movement. Because presenting treatments in the same order might lead to apparent differences based on treatment order rather than the treatments themselves, we derived our null probability from the results of the clean air assay. If each individual beetle had an innate tendency to move a random distance in the walking chamber every time it was exposed to clean air, then the probability of an individual moving a greater distance upstream during the second exposure to clean air than the first would be 0.5.

The effects of beetle sex, block, and their interaction on the probability of a beetle moving further during the second exposure to a clean airstream than during the first exposure during the clean air assay were analyzed by logistic regression. Model selection was carried out by forward, backward, and stepwise selection with an α of 0.05 (PROC LOGISTIC, SAS Institute Inc. 2004). To evaluate whether the results were equivalent to the expected value of 0.5, the 95% confidence interval of the probability of moving a greater distance during the second exposure was calculated.

The results of the host attraction assay were evaluated by logistic regression (PROC LOGISTIC, SAS Institute Inc. 2004). The original model included tree species,

block, beetle sex, and all the interactions. Significant effects ($\alpha = 0.05$) were selected by using forward, backward, and stepwise selection. The simultaneous 95% confidence intervals (reflecting a Bonferroni-adjusted α of 0.00833) (Kuehl 2000) of the probability that a beetle moved a greater distance during exposure to each of the six tree odors were generated to evaluate whether the observed probabilities were different from the probability of 0.5 expected from random movement. The fifteen contrasts among individual tree species were also conducted and evaluated with a Bonferroni-adjusted α of 0.0033 (Kuehl 2000).

Because we hypothesized that the magnitude of the difference in the distance the beetles moved during the control and treatment exposures might contain information about the attractiveness of the odor, a nonparametric ANOVA on the ranks of the differences of the distance traveled by each beetle in the treatment exposure versus the control exposure was also carried out (PROC RANK, PROC MIXED SAS Institute Inc. 2004). However, the qualitative results of the ANOVA were identical to the results of the logistic regression, and are not presented.

Results

Sample Size

To achieve 99% confidence ($\alpha = 0.01$) in detecting a 10% difference in beetle movement, we determined that approximately 126 individuals would be needed. We elected to run six blocks (120 females and 120 males for each potential host species) in case there was an effect of beetle sex.

Clean Air Assay

When both the first and second airstream that a beetle experienced had no tree odors, the probability of a beetle moving further during the second exposure did not vary by beetle sex or block. Forward, backward, and stepwise model selection methods gave the same result. The 95% confidence interval for the probability contained the expected null value of 0.5 (actual probability = 0.492, lower confidence limit = 0.420, upper confidence limit = 0.597). We concluded that beetle movement was not detectably different between the first and second exposure to clean airstreams. Therefore, we used a probability of 0.5 as the expected null value if the treatment odor did not affect beetle behavior for the rest of the experiment.

Host Attraction Assay

Potential host species and block affected the probability of moving further in the treatment airstream than the control airstream in the host attraction assays (tree species: $df = 5$, Wald $\chi^2 = 18.96$, $P = 0.0019$; block: $df = 5$, Wald $\chi^2 = 13.44$, $P = 0.0196$) (Fig. 3.2). In the analysis of the probabilities, there was no effect of sex or interaction among sex, block, or tree species. Forward, backward and stepwise selection methods arrived at the same regression model. When the confidence intervals of the probability of moving further in the treatment airstream were compared to the value of 0.5 derived from the clean air assays, beetles had a higher than expected probability of moving towards the source only when the stimulus was from paper birch (Table 3.1). Thus, the odors from conifer bark and phloem in this experiment did not make *O. erosus* behave differently than it did in a clean airstream, but the odor from paper birch bark and phloem made them more likely to move towards the source of the odor.

In the comparison among species of the probability of moving a greater distance in the second airstream, the tree species fell into two groups (Figure 3.2): one group consisting of balsam fir, tamarack, and white spruce and another consisting of paper birch (the attractive odor), tamarack, hemlock, red pine, and white spruce. Differences between individual species may occur even when neither is different from the null because the assay was designed to detect differences in probability of about 0.1. The difference between some treatments was greater than 10% even if neither treatment had a greater than 10% difference from the null. Therefore, the beetle responses to the odors of

some tree species could be distinguished from each other, but the responses to all of the odors except paper birch were not distinguishable from random movement.

Discussion

This study is part of a broader series of experiments aimed at predicting the establishment potential of a theoretically introduced population of *O. erosus* in eastern North America. Small populations of *O. erosus* utilize recently killed trees or broken branches or stems for reproduction (Mendel et al. 1985), so during establishment the beetles would be expected to colonize dead host material. For establishment to occur, *O. erosus* must contact, bore into, and reproduce in trees that can support the development of offspring. Lee et al. (2008) and Walter et al. (in review-a) showed that red pine and white spruce support beetle reproduction greater than the replacement rate. Adults in contact with the bark surface readily bored into red pine, white spruce, and balsam fir; boring in tamarack and eastern hemlock occurred with moderate frequency (Walter et al. in review-a).

Previous studies that assayed walking behavior with olfactometers in the laboratory have demonstrated that the responses were indicative of responses obtained from flying beetles in field-trapping experiments (Wood 1970, Browne et al. 1974, Guerrero et al. 1997, Macias-Samano et al. 1998, Hovorka et al. 2005), although exceptions do occur (Wood 1970). We do not believe that our olfactometer results can be used to predict the exact percentage of beetles that will be attracted to the odors of potential host species. However, we do assume that these results are representative of the behavior of flying beetles: an odor that elicits a random response from beetles walking in an olfactometer will elicit a random response from flying beetles.

In this olfactometer assay, none of the odors of conifer species stimulated movement of the beetle that differed from the movement in response to a clean airstream. However, the odor of one allopatric non-host angiosperm, paper birch, elicited a positive response from the beetles. Thus, it appears that the response of *O. erosus* to the bark and phloem volatiles may not increase encounters with the coniferous hosts and non-hosts beyond what would be expected through random chance; encounters with paper birch might be higher than predicted by chance if the beetle only uses odors to locate hosts.

There are a number of difficulties associated with laboratory assays of walking responses of host attraction by bark beetles. The proportion of the population that typically responds to host odors is very low (Byers et al. 1985, Gast et al. 1993), and high concentrations of host volatiles may be necessary to elicit a walking response (Byers et al. 1985). However, high concentrations of conifer monoterpenes may be repellent (Seybold et al. 2006). Studies that examine the effect of only major odor components, or of individual volatiles may miss important effect due to ‘minor volatile components’ or synergism among compounds (Seybold et al. 2006). We felt that understanding the response of *O. erosus* to potential host odors is important because of the potential risks associated with the beetle establishing in new areas of North America (Eglitis 2000, Seybold and Downing 2009). During establishment, beetle populations would probably not be high enough for significant aggregation to occur, so the proportion of a small population that can successfully select a host is important.

Our experiment was designed to circumvent some of the more common problems with laboratory assays of host odors. The large sample size employed in this study (>1400 individual beetles) ensures that we would be able to detect a proportion of the

population as small as 10% that responded to a tree odor; as many as 50% of *Tomicus piniperda* respond to volatile components of the odor of Scots pine (Byers et al. 1985). Few olfactometer experiments have quantified the magnitude of the detectable response. In addition, the use of bark and phloem rather than compounds released from a headspace sampling device ensure that the concentrations used in the assay should be neither too low nor too high. The direct use of bark and phloem also ensures that we would detect effects due to minor components or synergy.

The positive response of *O. erosus* to the angiosperm non-host paper birch was unexpected. Volatiles such as *trans*-conophthorin found in paper birch interfere with the pheromone response of *O. erosus* (Zhang and Schlyter 2004) and other bark beetles (Byers et al. 2000, Huber et al. 2001, Jactel et al. 2001, Zhang and Schlyter 2003). However, bark extracts from other *Betula* species have been reported to contain 2-methyl-3-buten-2-ol (Zhang et al. 2000), a component of the aggregation pheromone of *O. erosus* (Giesen et al. 1984). Other coniferophagous bark beetles employ olfactory (Schroeder 1992, Guerrero et al. 1997, Huber and Borden 2001, Jactel et al. 2001, Byers et al. 2004) and visual (Campbell and Borden 2006) non-host cues when selecting hosts, and it is possible that non-chemical cues would have prompted *O. erosus* to avoid birch. Alternatively, the response of *O. erosus* to paper birch, like the absence of a strong response to other tree species in this experiment, may arise because the beetle and the tree are naturally allopatric.

Host selection studies in quarantine conditions do not include the full complement of stimuli that insects might use to select hosts in the field, which may lead to false positive and false negative responses (Zwolfer and Harris 1971). Other stimuli such as

visual profiles, both color and shape, and odors from parts of the plant other than the bark and phloem are known to be used for host location by other bark beetles (Person 1931, Schroeder 1992, Campbell and Borden 2006, Zhang et al. 2007). Nevertheless, our results with regard to members of *Pinus*, *Picea*, and *Abies* correspond with a field cage study with cut logs where *O. erosus* accepted member of each genera (Chararas et al. 1982).

Many bark beetle larvae are capable of developing on a wider variety of hosts than are actually selected by adults for oviposition in the field, and host selection rather than developmental ability is considered the major determinant of the ecological host range of bark beetles (Sauvard 2004). A similar pattern is seen for other insects (Courtney and Kibota 1990, Bernays and Chapman 1994). Thus, we had expected that the set of tree species that elicited a positive response in the olfactometer would be a subset of the species that support larval development (Lee et al. 2008, Walter et al. in review-a). It appears that more tree species are acceptable to *O. erosus* in contact with the bark than can support larval development, and the developmental host odors do not elicit a strong response from the *O. erosus* even though at least one non-host is attractive.

Past olfactometer studies using bark beetles have used open arenas (Wood 1962, Wood and Bushing 1963, Browne et al. 1974, Elkinton et al. 1980, Byers et al. 1985, Teale and Lanier 1991, Teale et al. 1991, Gast et al. 1993, Warren et al. 1996, McPherson et al. 1997), wind tunnels (Akers and Wood 1989), odor-laden airstreams intersecting a beam of light (Borden et al. 1968, Moeck 1970, Hovorka et al. 2005), Y-tube designs (Zhang et al. 2007), four-arm olfactometers (Guerrero et al. 1997), and T-mazes requiring beetles to climb (Akers and Wood 1989) (Table 3.2). Walking olfactometers have been

considered more amenable to laboratory assays than designs that allow beetles to fly. Flying bark beetles tend to move in a straight line until they collide with the side of the arena regardless of whether a stimulus is present, though the direction that the beetles choose to take off might differ in the presence of positive stimuli (reviewed in Riffell et al. 2008).

Because odor-plume dynamics in a forest can be very complicated, and odor concentration might not be indicative of distance to the source (Moeck et al. 1981), we chose to use an enclosed olfactometer with no difference in odor concentration. During preliminary experiments, we observed a large variation in the tendency of *O. erosus* individuals to move in several different types of olfactometer, regardless of the stimulus presented (AJW, personal observations). We examined each individual's response to clean and odor-laden airstreams to remove the influence of individual tendency to move long or short distances. Although we did not find a repellent treatment in this experiment, another advantage of this olfactometer design is that repellency can be detected if beetles tend to move shorter distances in odor-laden versus clean airstreams.

There are two competing hypotheses concerning host tree location by bark beetles: random landing and primary attraction. The random landing hypothesis states that bark beetles land indiscriminately on anything in the environment with the correct visual profile, and that the host use decision is made based on cues received while in contact with the plant (Person 1931, Moeck et al. 1981, Tunset et al. 1993, Brattli et al. 1998, Pureswaran and Borden 2005). The primary attraction hypothesis states that bark beetles use pre-landing olfactory information to guide their host selection, although post-landing information may also be used (Williamson 1996). Both of these hypotheses

implicitly apply to the interaction between bark beetles and evolutionarily sympatric hosts. When presented with odors from a set of allopatric tree species, *O. erosus* discriminates among species, although distinguishing this behavior from random landing in the field would be difficult. The apparent inability of *O. erosus* to identify potential host based on odors has at least three explanations: (i) *O. erosus* may not respond to tree odors during host selection, (ii) the beetles may require other cues, such as visual stimuli in combination with olfactory stimuli to select a host, or (iii) *O. erosus* may not be able to discriminate hosts from non-host when there has been no history of association with the plants.

Invasive insects are a source of major environmental and economic damage in North America and throughout the world, but many species that are introduced do not become damaging invaders (Lee et al. 2005). *Orthotomicus erosus* has established a small population in North America, but its potential to cause widespread damage is poorly understood (Wood 1962). Studying the response of this beetle to allopatric potential host plants under quarantine conditions helps to clarify the risk posed by the beetle. *Orthotomicus erosus* responds positively to the odor of an allopatric angiosperm non-host but does not respond to the odors of several allopatric host and non-host conifers in a way that can be distinguished from random movement, although its response to some conifer species can be distinguished from its response to others. If the beetle's geographic range expanded to coincide with these tree species, it does not appear that the beetle could use bark and phloem odors alone to distinguish between species that would and would not support reproduction.

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Table 3.1. Lower and upper bound of the confidence interval probability of the response of the Mediterranean Pine Engraver, *Orthotomicus erosus*^a

Species	Probability of moving a greater distance when exposed to treatment odor ^b			Observed Value	N
	Lower Bound	Upper Bound			
Red Pine	0.4908	0.7119		0.61	234
White Spruce	0.4561	0.6800		0.57	239
Tamarack	0.4763	0.6977		0.59	240
Eastern Hemlock	0.4848	0.7051		0.60	240
Balsam Fir	0.3476	0.5732		0.46	240
Paper Birch *	0.5207	0.7281		0.63	276

^aProbability that an individual would walk a greater distance in the olfactometer when presented with an airstream containing volatiles from the bark and phloem of the six tested tree species than in a clean airstream.

^bTo control the experimentwise α at 0.05, a Bonferroni-adjusted α of 0.0083 was used for confidence interval calculations. Results marked with an * are significantly different from the value of 0.5 expected from random movement.

Table 3.2. Previous studies of bark beetle response in walking olfactometers, the stimuli tested, and olfactometer type.

Species	Stimuli tested	Olfactometer Type	Reference
<i>Dendroctonus pseudotsugae</i>	Sex pheromone	Open arena	Borden et al. 1968
<i>Dendroctonus valens</i>	Non-host volatiles	Y-tube	Zhang et al. 2007
<i>Hylastes ater</i>	Host, non-host and congeneric beetle odors	4-arm	Leahy et al. 2007
<i>Ips paraconfusus</i>	Aggregation pheromone	Open arena	Wood 1962
<i>Ips paraconfusus</i>	Aggregation pheromone	Open arena	Wood and Bushing 1963
<i>Ips paraconfusus</i>	Aggregation pheromone	Open arena	Elkinton et al. 1980
<i>Ips paraconfusus</i>	Aggregation pheromone components	Wind tunnel	Akers and Wood 1989
<i>Ips paraconfusus</i>	Aggregation pheromone, verbenone	Open arena	McPheron et al. 1997
<i>Ips pini</i>	Aggregation pheromone	Open arena	Browne et al. 1974
<i>Ips pini</i>	Pheromone component	Open arena	Teale and Lanier 1991
<i>Ips pini</i>	Pheromone component	Open arena	Teale et al. 1991
<i>Ips pini</i>	Host odor, Aggregation pheromone	Open arena	Gast et al. 1993
<i>Ips plastographus maritimus</i>	Host odors, sex pheromone	Open arena	Warren et al. 1996
<i>Scolytus intricatus</i>	Host odors, aggregation pheromone	Airstream intersecting a beam of light	Hovorka et al. 2005
<i>Tomicus destruens</i>	Non-host volatiles	Climbing	Guerrero et al. 1997
<i>Tomicus destruens</i>	Host volatiles	4-arm	Faccoli et al. 2008
<i>Tomicus piniperda</i>	Host odors and volatiles	Open arena	Byers et al. 1985
<i>Tyrpodendron lineatum</i>	n/a	Airstream intersecting a beam of light	Moeck 1970

Fig. 3.1. Schematic of laboratory olfactometer. Air flowed from the pump through the hydrocarbon filter and then through the humidifier. Next, the airstream was split, with each half going to a flowmeter set at 400 ml/min, then into an odor chamber, empty or with bark and phloem from one of the tested tree species. After leaving the odor chamber, each airstream was split again before entering walking chambers inside the white box. All connections prior to the walking chambers were made with PTFE tubing. After the airstream had been split, identical components of the olfactometer were connected by using tubing of the same length.

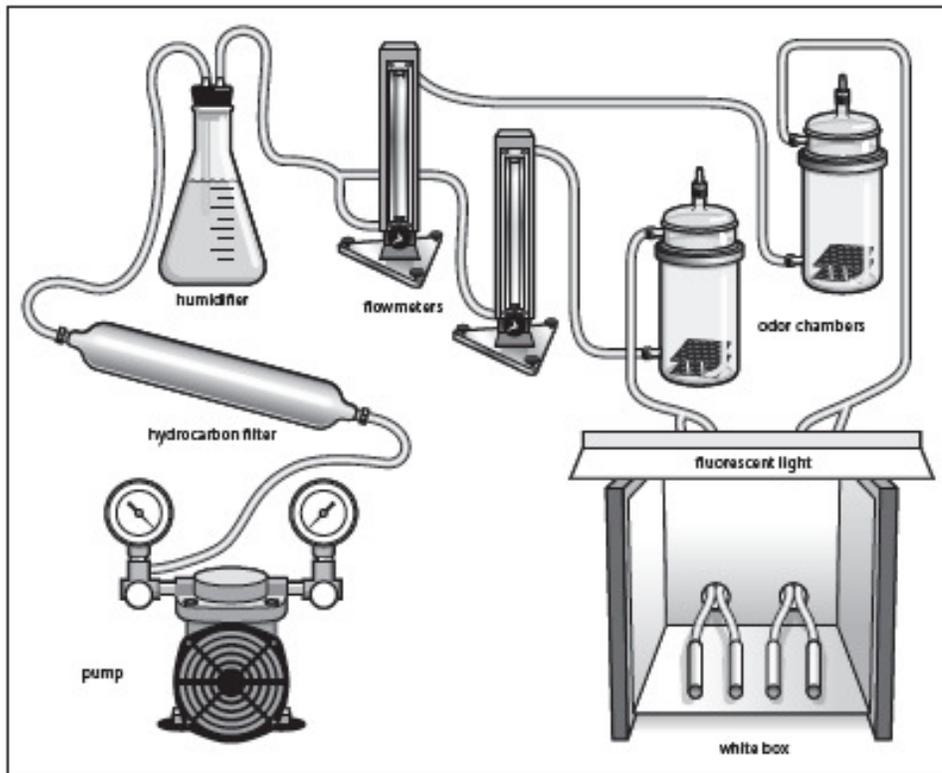
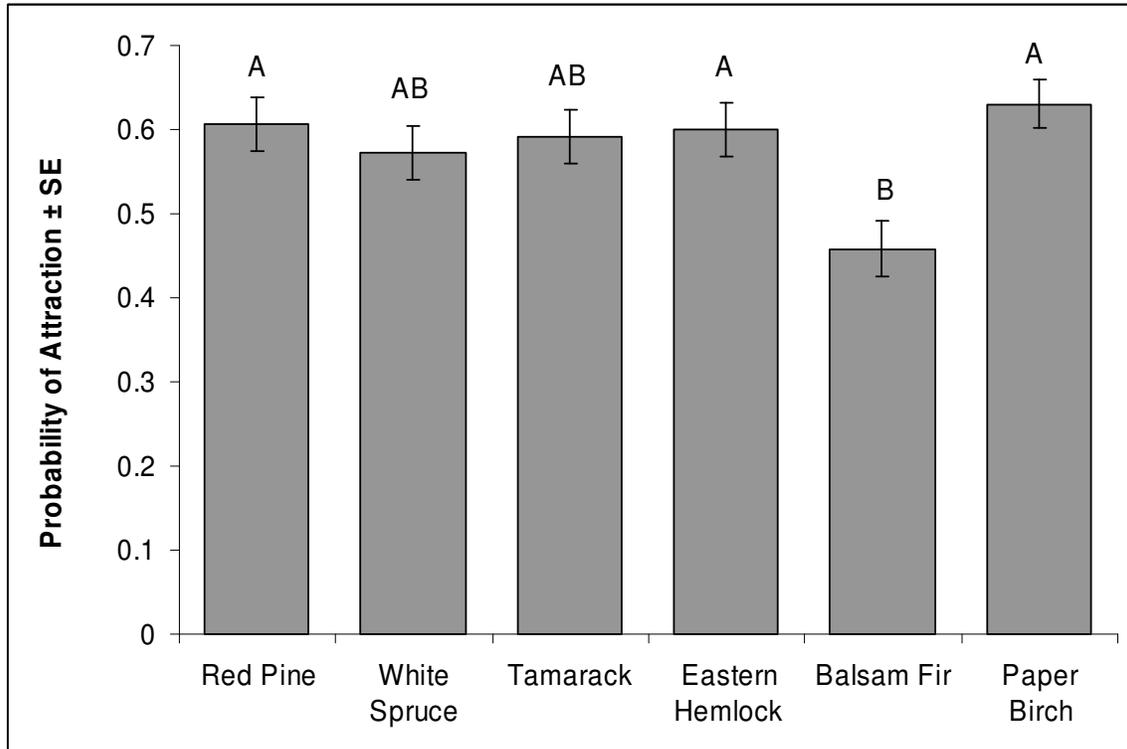


Fig. 3.2. Probability that individual *Orthotomicus erosus* moved further in a straight tube when exposed to an airstream containing odor from the bark and phloem of selected tree species than when exposed to a clean airstream. Bars labeled with the same letter were not significantly different according to a contrast by tree species in logistic regression.



**Chapter 4. Allopatric host and non-host plants contain feeding stimulants for the
Mediterranean pine engraver, *Orthotomicus erosus* (Coleoptera: Scolytidae)**

Summary. When invasive herbivorous insects enter a new environment and encounter previously allopatric plant species, they must rely on positive and negative stimuli to determine if these plants might be hosts. For the Mediterranean pine engraver, *Orthotomicus erosus* (Wollaston), a bark beetle poised to expand its range in North America, stimuli encountered from tree bark appear to be determinants of host selection. We prepared bark and phloem extracts with water, methanol, and hexane from four eastern North American tree species, which are currently allopatric to an introduced population of the beetle. Extracts were presented on a neutral feeding substrate alone and in combination with each other. Methanol extracts of paper birch, *Betula papyrifera* Marsh., balsam fir, *Abies balsamea* (L.) Mill, tamarack, *Larix laricina* (Du Roi) Koch, and red pine, *Pinus resinosa* Aiton and the aqueous extracts of *B. papyrifera* and *L. laricina* incited (increased the initiation of) feeding. The aqueous extract of *L. laricina* also stimulated (increased the extent of) feeding. The effects of extracted chemicals reported here do not match the observed behavior of the beetles on the same tree species, indicating that host selection by the beetle may not be predictable from bark and phloem chemistry.

Key words. Allopatry – Coleoptera – feedant – feeding incitant – feeding stimulant – gustation – novel plant association – Pinaceae – Coleoptera – Scolytidae

Introduction

An herbivorous insect uses positive and/or negative stimuli to determine whether to use plants encountered in its environment as hosts. A given host-associated behavior, such as oviposition, may require that certain positive stimuli be present or negative stimuli be absent (Thorsteinson 1960). The presence of negative stimuli, which would be encountered in most plants by most insects, may be an especially important determinant of the host range of oligophagous insects (Jermy 1966, Schoonhoven et al. 1998).

The Mediterranean pine engraver, *Orthotomicus erosus* (Wollaston) (Coleoptera: Scolytidae, sensu Wood 2007) is a bark beetle recently detected in North America. Presently, it exists as a small established population in California (Lee et al. 2005, Seybold and Downing in press). The beetle has been reported in association with a wide phylogenetic range of conifers from the families Pinaceae and Cupressaceae in its native and adventive geographic range (Mendel and Halperin 1982, Wood and Bright 1992, Bright and Skidmore 1997, Lee et al. 2008). Therefore, there is concern that many North American conifer species will be attacked if the beetle population expands eastward. A number of species in the genera *Pinus*, *Picea*, and *Pseudotsuga* are suitable for the development of the beetle (Lee et al. 2008, Walter et al. in review-a). When confined on the bark, the beetle bores into both suitable and unsuitable species (Walter et al. in review-a), but tree odors from the same set of species do not elicit different behavior than would be expected by chance in a walking olfactometer (Walter et al. in review-b).

Predicting the host range of an insect is a difficult problem, but is necessary for management because individually testing each member of large groups such as Pinaceae

for every potentially invasive organism is not practical. If the criteria that cause a plant to be treated as a host or non-host are heritable, then insect host range should be predictable from plant phylogeny. Plant phylogeny has been used to determine the species to be included in pre-release safety testing for candidate agents of classical biological control (Wapshere 1974). However, some plant traits known to be of great importance to insects, such as secondary compound chemistry and suites of defensive traits, do follow the same pattern as the phylogeny (Becerra 1997, Agrawal and Fishbein 2006). In-depth knowledge of the mechanism of host selection is needed. For insect species where the plant traits relevant to host selection are known, this knowledge could be used directly to predict insect response. For species where the traits are unknown, it may be possible to assess whether the relevant traits are likely to be associated with a known character, such as phylogenetic relatedness or habitat. The known character could then be used as the basis for prediction.

For insects with sedentary larvae, such as bark beetles, the ecological host range will be determined by the adults that select the plant where the eggs are deposited (Schaffner 2001). Knowledge of the plant characters that drive adult selection behavior may allow improved prediction of insect host range. Previous work has shown that adults of *O. erosus* bore into the outer bark of several potential host species at different rates, although other stages of host selection such as olfactory attraction and gallery abandonment are similar between the species (Walter et al. in review-a, Walter et al. in review-b). Furthermore, a number of tree-derived feedants (Doskotch et al. 1969, Levy et al. 1974, Meyer and Norris 1974, Raffa and Berryman 1982, Eriksson et al. 2008) and antifeedants (Norris and Baker 1967, Gilbert and Norris 1968, Salom et al. 1994, Klepzig

et al. 1996, Wallin and Raffa 2000, 2002a, Faccoli et al. 2005, Mansson et al. 2005, Eriksson et al. 2008) are known from other bark beetles. We are searching for the presence of chemicals that affect feeding initiation (feeding incitants and suppressants) or the extent of feeding (feeding stimulants and deterrents) (terminology from Beck 1965). We have focused on the response of the beetles to chemical stimuli encountered after coming into contact with the bark because by extracting chemicals and applying them to a new substrate, the effect of chemicals can be separated from other host traits such as bark texture or visual profile that are also known to be important (Elkinton and Wood 1980, Campbell and Borden 2006) but more difficult to characterize.

We hypothesized that the final behavioral events in host acceptance by *O. erosus* would take place on the basis of chemical compounds in the bark and phloem of potential host species. Under this hypothesis, the species where the beetle has the highest probability of boring should contain chemicals with feedant (incitant or stimulant) effects, and species where the beetle has low boring activity would contain antifeedant (suppressant or deterrent) compounds.

Materials and Methods

Location of research

Work with live insects took place in the Minnesota Agricultural Experiment Station / Minnesota Department of Agriculture Biological Level 2 Containment Facility in St. Paul, MN. *Orthotomicus erosus* was collected in California (see below) and from a colony maintained in St. Paul. All handling procedures were approved by USDA APHIS Plant Protection and Quarantine Division (Permit 74447).

Field collection of beetles and logs for gustation assays

Cut logs of Italian stone pine, *Pinus pinea* L., and Aleppo pine, *Pinus halepensis* Mill., infested with *O. erosus* were collected in Fresno County, CA in summer 2008 and held in emergence boxes (Browne 1972). Adults were allowed to emerge naturally and were kept in refrigerators (approx. 10 °C) in jars with moist paper towel until shipment. On 13 August and 7 October 2008, emerged beetles were shipped to St. Paul, MN in insulated styrofoam boxes with ice packs. All beetles were held in a commercial refrigerator at approximately 10° C and deprived of food until used in the experiment.

Tree species for the gustation assays were: red pine, tamarack (*Larix laricina* (Du Roi) Koch), balsam fir (*Abies balsamea* (L.) Mill.), and paper birch (*Betula papyrifera*

Marsh.). Two trees of each test species (28-53 cm diameter) were felled at the University of Minnesota Northern Research and Outreach Center (Grand Rapids, MN) between 16 and 19 June 2008 and cut immediately to approximately 50 cm lengths. Cut surfaces were sealed with paraffin wax and stored in a greenhouse with a minimum temperature of 7-13°C during the day and 4-10°C at night.

Beetle colony

A colony of *O. erosus* was maintained on bolts of red pine, *Pinus resinosa* Aiton (approximately 50 cm x 15 - 40 cm diam.) in a growth chamber at 25°C, 16:8 L:D as described previously (Walter et al. in review-b).

Chemical Extraction

Chemical extractions were performed on 4 August 2008 (2 *Pinus resinosa*, 1 *Abies balsamea*, 1 *Betula papyrifera*), 20 August 2008 (2 *Larix laricina*, 1 *Abies balsamea*, 1 *Betula papyrifera*), and 23 September 2008 (1 *Pinus resinosa*, 1 *Larix laricina*, 1 *Abies balsamea*, 1 *Betula papyrifera*) 2008. In all, two sets of extracts were made from one tree of each species, and one set of extracts was made from the other. For each extraction, three sets of two 5 cm diameter discs were cut through the outer bark and phloem of bolts with a hole saw attached to a power drill; the discs were removed with a

draw knife. The discs were cut into strips a maximum of 1 cm wide and placed into 250 ml Erlenmeyer solvent flasks. We added 200 ml of solvent to each flask; the flasks were shaken at 100 rpm at room temperature for 48 hr, and the solvent and extracted chemicals were decanted into clean 500 ml amber glass jars. The solvents were water (HPLC Grade water, Fisher Scientific, Fair Lawn, NJ), methanol (HPLC Grade methanol, Fisher Scientific), and *n*-hexane (Environmental Grade, 95%, Alfa Aesar, Ward Hill, MA). Between extraction and use in the experiment, the hexane and methanol extracts were stored in the dark in a flame-proof cabinet at room temperature, and the water extracts were stored in a dark growth chamber at 4°C to minimize decomposition by microbes.

Behavioral Assay

The experiment followed a factorial design for each tree species with two levels of each of four factors: 1) beetle sex (male or female); 2) methanol extract (present or absent); 3) hexane extract (present or absent); and 4) water extract (present or absent). Bark and phloem for the extracts for each species were taken from logs of two individuals per species. Treatments were presented on a neutral feeding substrate (Fisherbrand P5 qualitative filter paper, Fisher Scientific, Pittsburgh, PA). The extract was applied to the presence treatment and the solvent was allowed to evaporate. Nothing was applied to the substrate in the absence treatment. Each combination was replicated 3 times in each of 7 blocks. We ran 3 replicates of 6 additional treatments consisting of male or female beetles presented with substrate that had received clean methanol, hexane, or water from

the same lots as our extraction solvents in each block. A total of 1344 individual beetles (32 treatments with 3 males and 3 females per block in 7 blocks) was used in the experiment.

Strips (2.5 cm x 15 cm) of filter paper were used as neutral feeding substrates for the beetles. The filter paper strips used in this study had a mass equivalent to about 1.5% of the red pine sample used in each 200 ml extraction. Therefore, solvents were applied to the strips in 3 ml aliquots, so that the amount of extracted chemicals per unit mass in the experiment would be approximately equivalent to what the beetles would encounter from trees. After the solvents were applied to the paper, the paper was placed on clean aluminum foil while the solvent evaporated in a fume hood. If more than one solvent was used in a treatment, one extract was placed on the paper and the solvent was allowed to completely evaporate before the next extract was added. After all the solvents had evaporated, the paper strips were rolled into a 2.5 cm long cylinder by a technician wearing nitrile gloves, and inserted into 0.60 cm interior diameter plastic drinking straws (Diamond flexible straws, Jarden Home Brands, Muncie, IN) cut to approximately 8 cm lengths to create feeding arenas that simulated bark beetle galleries. Some bark beetles, such as *Ips typographus*, will not feed unless the feeding substrate is presented in an artificial gallery (Schlyter et al. 2004). Our preliminary experiments showed this was also true for *O. erosus*.

The filter paper substrates were moistened to the approximate moisture content of phloem of *Picea abies* (L.) Karsten (1.4-2.6 mg / mg dry weight, Gall et al. 2002) to encourage beetle feeding. Inside the quarantine facility, one end of each arena was sealed with white laboratory tape (Timemed labeling tape, Fisher Scientific,). An aliquot (0.7

m) of distilled deionized water was placed in the open end of the arena, and the water was allowed to soak into the paper for at least two hours. Excess water was shaken out of the straw. A beetle was placed in the straw, and the open end was sealed with white laboratory tape. The straws were placed vertically in plastic cups in a growth chamber (25°C, 16:8 L:D) for approximately 72 hr.

At the end of the experiment, all beetles were removed from the straws, and straws were frozen for at least 24 hr at -80°C before being removed from the quarantine lab. The amount of paper eaten was determined in a method analogous to O'Neal et al. (2002). The paper strips were removed from the straws and examined for feeding. Papers with detectable feeding were scanned on a flatbed scanner (HP Scanjet 4890, Hewlett-Packard, Palo Alto, CA). A rectangle of the scanned black and white image was highlighted with the polygonal lasso in Adobe Photoshop 7.0 (Adobe, San Jose, CA); the percentage of black in the area was determined using the histogram tool, and the dimensions of the rectangle were determined using the cut-paste tool. From this information, the area of the filter paper that was consumed by the beetle was determined.

Data Analysis

The results of each tree species were analyzed separately. To determine whether tree individuals and extraction dates affected beetle behavior, initial analyses including block, beetle sex, tree and extraction, and treatment was conducted for feeding initiation and the extent of feeding. Feeding initiation was analyzed by logistic regression and the extent of

feeding was analyzed by a mixed model ANOVA as described below. The probability that tree and extraction date or their interaction with any other effect affected feeding initiation or the extent of feeding was not significant ($P > 0.05$) in all cases. Therefore, tree individuals within each species and time of extraction were pooled for the remainder of the analysis.

Both feeding initiation and the extent of feeding were analyzed in factorial analyses. Because the presence treatment for each extraction included extracted chemicals applied in the extraction solvent and the absence treatment had nothing applied, the effects of the extracted chemicals cannot be separated from the effects of applying the solvents to the filter paper in a single analysis. To separate the effect of applying the solvents to the papers and allowing them to evaporate from the effect of the extracted chemicals, the effect of applying each solvent to the filter paper was estimated from analyses of feeding initiation and the extent of feeding in the treatments where only the clean solvents were applied. The confidence intervals of the difference between the parameters from the solvent only analysis and the factorial analysis for the extracts of each species were then compared. If the confidence interval of the difference did not contain zero, the chemicals contained in the extracts significantly affected beetle behavior.

Feeding Incitants and suppressants

A feeding incitant should increase the probability of feeding initiation by an insect while a feeding suppressant should reduce the probability of feeding initiation. Feeding initiation was analyzed by logistic regression (PROC LOGISTIC, SAS Institute Inc. 2004). The full model contained 1) block, sex, methanol extract, hexane extract, water extract and all possible interactions for the tree species and 2) block, sex, methanol, hexane, water and all possible interactions for the blanks. The model was reduced by forward, backward and stepwise selection with $\alpha = 0.05$. Forward and stepwise selection always yielded the same model, but backward selection tended to return models with more terms, probably because of a greater probability of type I error associated with backward selection methods. For this reason, the model returned by forward and stepwise selection was used. The main effects of the three extracts (methanol, hexane, and water effects) were forced into the model regardless of whether they were returned by forward and stepwise selection. For each analysis the confidence intervals for the intercept, methanol (extract), hexane (extract), and water (extract) were estimated at an α level of 0.00125, reflecting a Bonferroni-adjusted overall α of 0.05 (Kuehl 2000).

Feeding stimulants and deterrents

A feeding stimulant should increase the amount of feeding by an insect that has begun feeding while a feeding deterrent should decrease the amount of feeding that occurs after

an insect has begun. For those cases where feeding was initiated, the extent of feeding was analyzed by a mixed model ANOVA (PROC MIXED, SAS Institute Inc. 2004). Because the data did not meet assumptions of normality or homoscedasticity, the area removed by the beetles was taken to the power of 0.15 as suggested by a Box-Cox transformation (Arc version 1.06 software package, www.stat.umn.edu/arc/). Following the transformation, which corrects for homoscedasticity, all the datasets were retested for normality (PROC UNIVARIATE, SAS Institute Inc. 2004). All transformed datasets met the assumption of normality. The full model contained block as a random effect and sex, methanol extract, hexane extract, water extract, and all possible interactions as main effect for the tree species and block as a random effect and sex, methanol, hexane, and water for the blanks. The main effects were reduced by backwards selection (Weisberg 2005) with $\alpha = 0.05$, with the main effects of the three solvents (methanol, hexane and water effects) forced into the model. The confidence intervals for the three solvent types and any other effects included in the final model was generated with a Bonferroni-adjusted overall α of 0.05. In one tree species, there was a significant interaction between two types of extractions. The estimated parameter for this interaction was compared with the sum of the parameters for the corresponding clean solvents.

Results

Feeding Incitants

The models generated by forward and stepwise selection included effects of block in all five datasets. Interactions between the effects were included in the final models, indicating that the extracted materials in the three solvents did not act synergistically. Parameters were estimated for the effect of all three solvents or extracts regardless of whether they met criteria for inclusion in the model. The methanol extract of *Betula papyrifera*, *Abies balsamea*, *Larix laricina*, and *Pinus resinosa* as well as the water extracts of *Betula papyrifera* and *Larix laricina* incited feeding. (Fig. 4.1). Because of the factorial analysis, direct mean comparisons between treatments are not appropriate, but an examination of the mean and standard error of the treatments for each species shows a qualitatively higher probability of feeding initiation on papers treated with treatments treated with the methanol extract of all four species water extracts of paper birch and tamarack (Fig. 4.2).

Feeding stimulants

In addition to the main effects for the three solvents that were forced into the models, the final models generated for *Abies balsamea* included the methanol extract * sex interaction and the hexane extract * water extract * sex interaction and all sub-effects of those interactions and the *Larix laricina* model included the main effect of sex. For the

blank solvents, three separate sets of parameters were estimated so that the effect of the extracted chemicals and their solvents could be compared to the effect of solvents alone for each of the three models used to analyze the species datasets. The significance of the hexane extract * water extract interaction in the balsam fir dataset was evaluated by comparing it to the sum of the effects of the hexane solvent and the water solvent in the blank treatments. The only behaviorally active extract was the water extract of tamarack, which was a feeding stimulant (Fig. 4.3). As with the feeding initiation results, direct comparison of means is inappropriate, but examination of the treatment means and standard errors shows no qualitative differences between the treatments (Fig. 4.4).

Discussion

We found that the methanol extracts of all four species and the water extracts of paper birch and tamarack incited feeding by *O. erosus*. The water extract of tamarack also stimulated feeding. These results are different from previous behavioral studies that showed that the probability that *O. erosus* will bore into the outer bark is highest for red pine and balsam fir, intermediate for tamarack, and lowest for paper birch (Walter et al. in review-a). Other sources of information, for example visual or tactile cues, may be important in the host acceptance decision of *O. erosus*.

Insects confined to a testing arena may become habituated to negative stimuli, or more likely to feed on a substrate with a low level of stimulants because of hunger or thirst. The long assay time used in this experiment (72 hr) increases the probability that increased feeding will occur in response to even a slight feedant but decreases the possibility that antifeedant activity will be detected. In a similar experiment with Pales weevil, *Hyllobius pales* (Herbst.), some compounds with statistically significant antifeedant activity after the beetles had been exposed for 24 hr were not active antifeedants when the beetles had been exposed for 48 hr (Salom et al. 1994). Long duration no-choice tests are considered appropriate in host-range testing for biological control agents, and by extension for other insects entering new geographic areas. In these situations, the most preferred host of an insect might not be available in the field, but insects would encounter novel plants until they either accepted a novel plant or died (Tallamy 2000, Barton Browne and Withers 2002).

Polar and non-polar extracts of the bark of their normal host plants have activity for a number of conifer- and angiosperm-feeding bark beetles. Methanol extracts from the host plants of *Dendroctonus ponderosae* Hopkins, *D. frontalis* Zimmermann, *Ips paraconfusus* Lanier, and *Coccotrypes dactyliperda* (Fabricius) incite feeding (Elkinton et al. 1981, Thomas et al. 1981, Raffa and Berryman 1982, Meisner et al. 1985, McNee et al. 2003). Feeding in *D. ponderosae*, *D. frontalis*, *Ips paraconfusus*, *Scolytus mediterraneus* (Eggers), and *S. multistriatus* (Marsham) is also incited by non-polar benzene or ether extracts of their hosts (Norris and Baker 1967, Levy et al. 1974, Elkinton et al. 1981, Thomas et al. 1981, Raffa and Berryman 1982, McNee et al. 2003). In addition to the effect of methanol and ether extracts, feeding by *I. paraconfusus* is stimulated by water and the combined water and ether extracts of *Pinus ponderosa* C. Lawson (Elkinton et al. 1981, McNee et al. 2003). Antifeedant activity from host plant extracts was not reported in these studies, although extracts of diseased or damaged host trees have been reported to have antifeedant activity relative to healthy host trees for *I. paraconfusus*, *I. pini* (Say), and *Hylastes porculus* Erichson (Klepzig et al. 1996, McNee et al. 2003).

A number of compounds known to be present in host plants, especially monoterpenes and phenolics, are antifeedants when tested individually at high concentrations typical of induced plant defense (Klepzig et al. 1996, Wallin and Raffa 2000, 2002a, Faccoli and Schlyter 2007). This experiment was designed to assay feedant and antifeedant compounds in uninduced, recently killed trees because we consider these trees to be the most likely hosts for a small population of invading bark beetles. This study would not show antifeedant effects due to the induction of defenses because the

trees used in the study did not appear to be under herbivore or pathogen attack when they were cut. The tamarack trees used in this study had far less resin than similarly sized trees under attack by the eastern larch beetle, *Dendroctonus simplex* LeConte, when they were felled (AJW, personal observation).

Most of the extracts with feedant activity in this experiment were from tree species that do not support the reproduction of *O. erosus* (Lee et al. 2008, Walter et al. in review-a). Although various non-host chemicals have been tested for gustatory activity individually for a variety of bark beetle species (Gilbert and Norris 1968, Salom et al. 1994, Faccoli et al. 2005), only a few studies on angiosperm-feeding bark beetles have examined bark extracts from non-host trees. Four out of ten non-hosts of *Hylobius abietis* (L.) and four non-hosts of *S. multistriatus* had feeding stimulants in non-polar extracts (Gilbert et al. 1967, Eriksson et al. 2008). Polar extracts of four out of the same ten non-hosts of *H. abietis* and one non-host of *S. multistriatus* had feeding deterrent activity (Gilbert and Norris 1968, Eriksson et al. 2008).

The presence of feeding incitants in polar and aqueous extracts of tree species in this study is similar to pattern found in sympatric coniferous host plants of several bark beetle species. In some cases, bark beetles may be able to avoid non-host trees, even if they contain feeding incitants, based on other cues such as repellent volatiles (Schroeder 1992, Guerrero et al. 1997, Huber and Borden 2001, Jactel et al. 2001, Byers et al. 2004, Graves et al. 2008). However, the response of *O. erosus* to odors from the four tested tree species in an olfactometer could not be distinguished from random movement (Walter et al. in review-b). Because all of the tree species tested are currently allopatric to the distribution of the *O. erosus*, there is no *a priori* reason the beetle should be able to

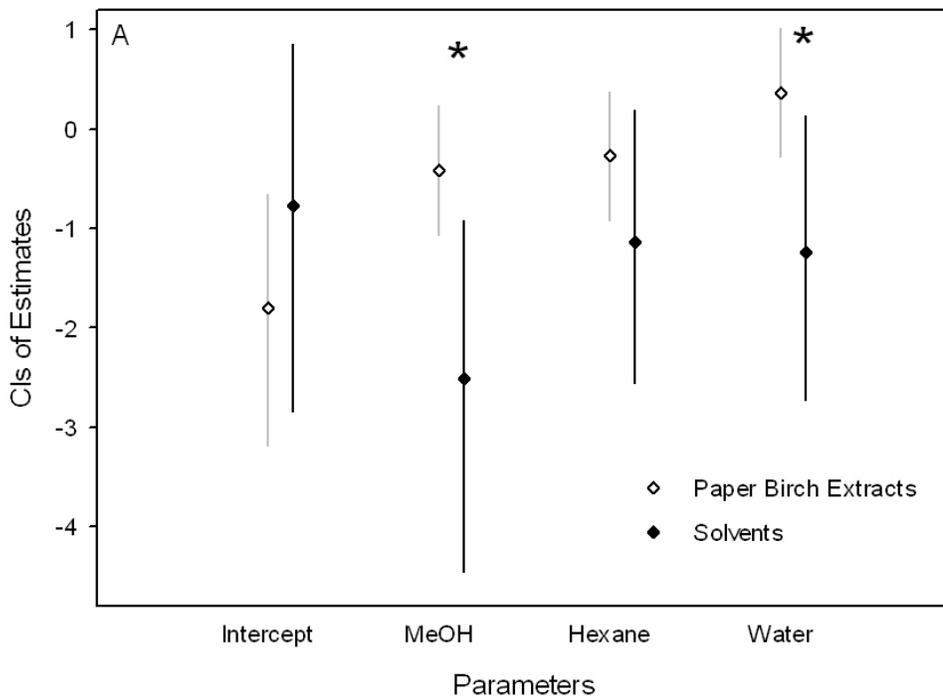
make a “proper” colonization decision based on the stimuli contained in these plants. Insects that encounter previously-allopatric invasive plant species (Graves and Shapiro 2003, Casagrande and Dacey 2007) and schistosome parasites that encounter allopatric snail species (Allan et al. 2009) will select hosts for oviposition that do not support the development of their larvae. When the insect is the invader, this should be expected to occur frequently because the selection threshold of insects that do not encounter a feeding or oviposition cue should become lower and lower until the insect attempts to use something in the novel environment (the hierarchy-threshold model, Courtney and Kibota 1990).

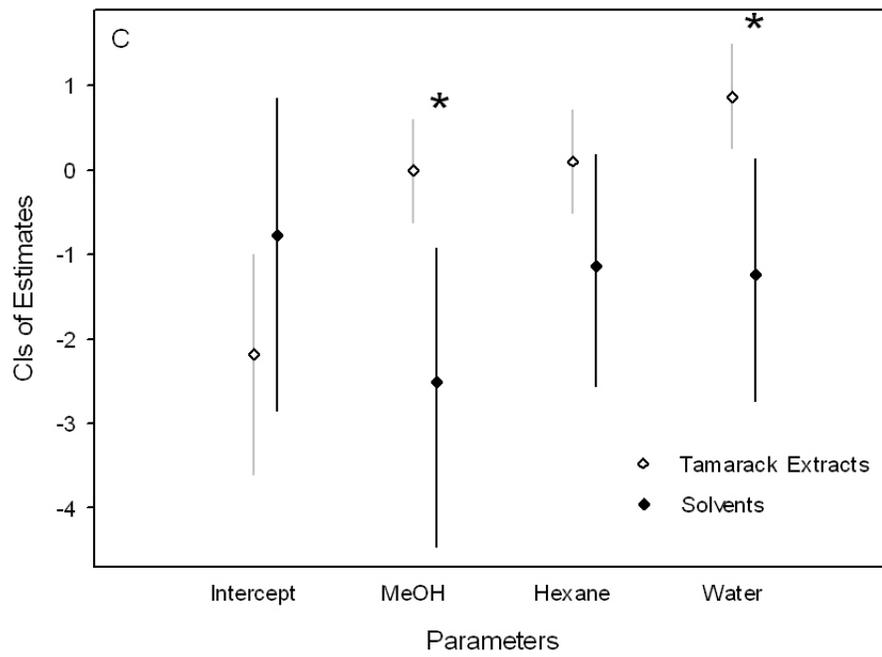
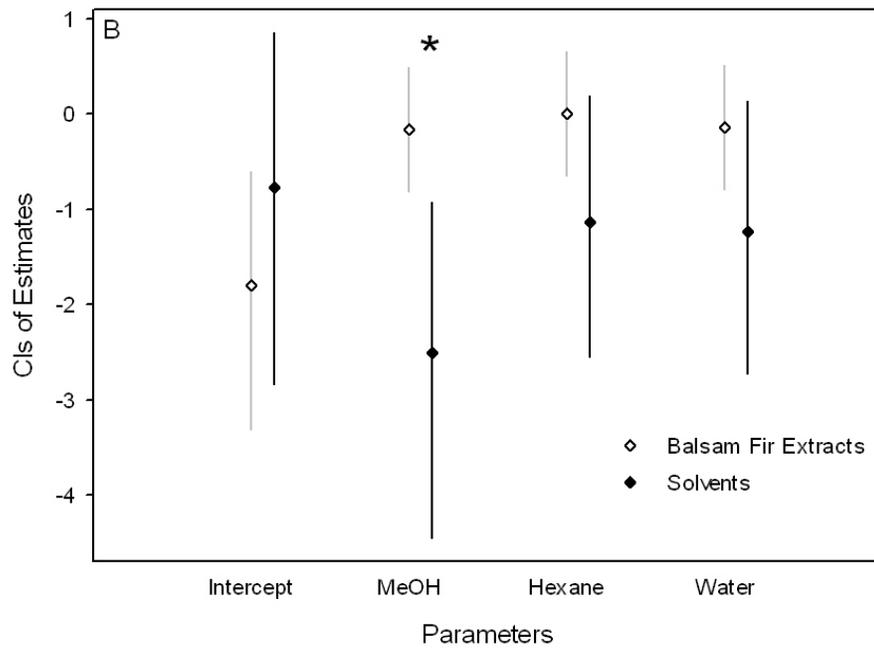
When an insect encounters a plant, it decides whether to attempt to use the plant as a host based on a number of chemical and non-chemical stimuli. We tested gustatory stimuli from several allopatric host and non-host plants of *O. erosus*, and we found feeding incitants in all four species, and feeding stimulants in one species. The feeding behavior of *O. erosus* exposed to bark and phloem extracts in this study did not match the behavior of the beetle when exposed to the bark and phloem of the same tree species, indicating that non-chemical cues may be important in the host-use decision of this beetle. This indicates that bark and phloem chemistry will not be a useful tool in making host range predictions for *O. erosus*.

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Fig. 4.1. Simultaneous 95% confidence intervals (CIs) of parameter estimates of feeding initiation for each species from the logistic regression models of the effect of each extract from A) *Betula papyrifera*, B) *Abies balsamea*, C) *Larix laricina*, and D) *Pinus resinosa* and the corresponding solvents on feeding initiation by *Orthotomicus erosus*. Cases where the difference of the parameter value of the extracts and the corresponding solvent does not overlap with zero are marked with a *. A higher value of response elicited by the extract than the blank denotes feeding incitant activity, whereas a lower value of response elicited by the extract denotes feeding suppressant activity.





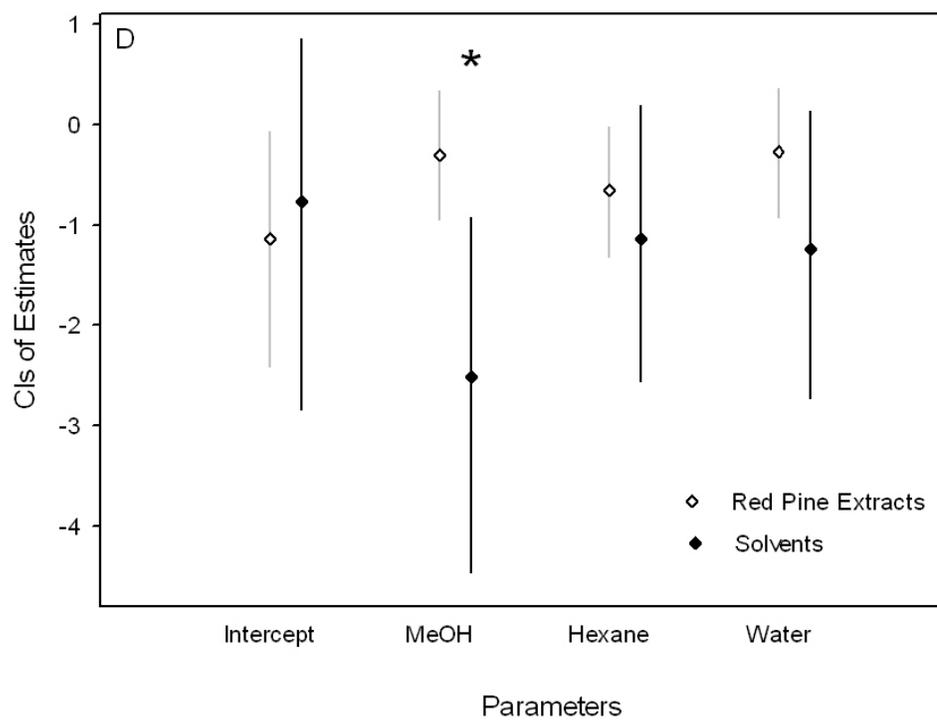
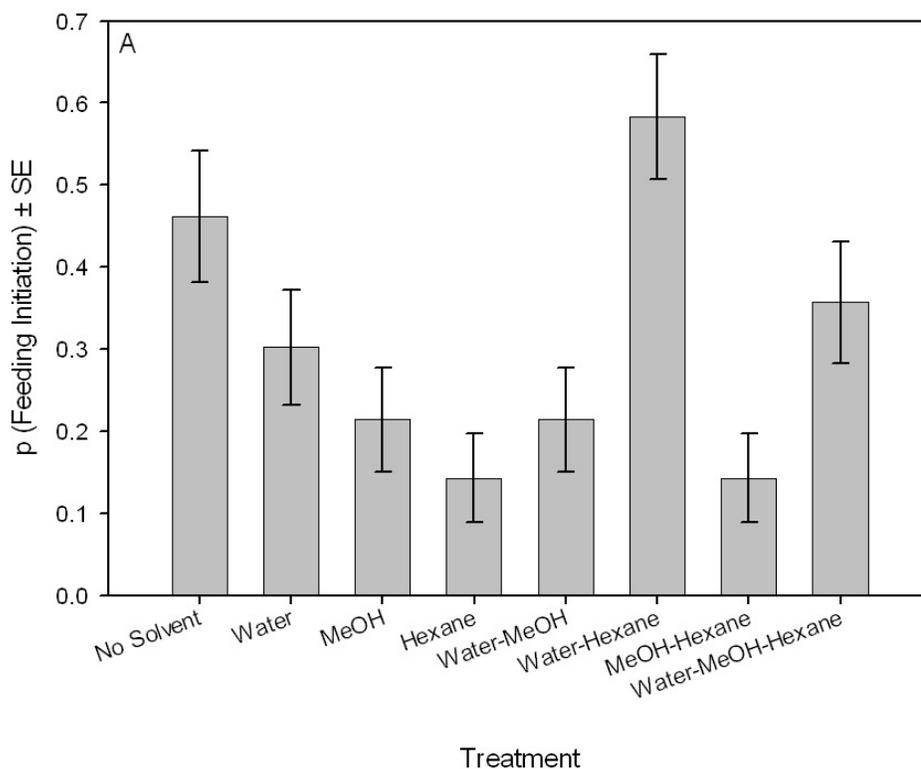
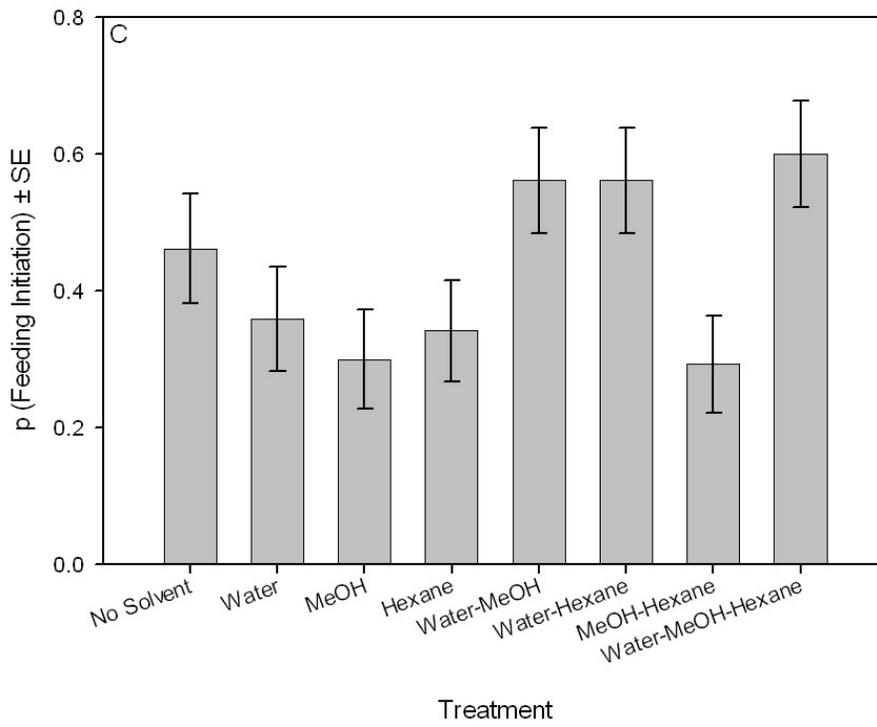
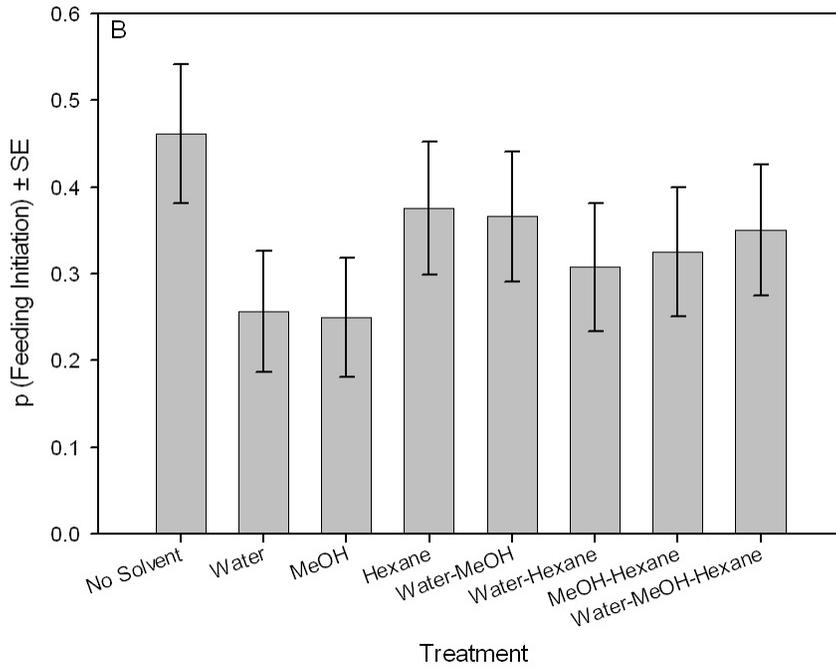


Fig. 4.2. Mean \pm binomial standard error (SE) of the probability of feeding initiation on filter paper containing single and combined extracts of A) *Betula papyrifera*, B) *Abies balsamea*, C) *Larix laricina*, D) *Pinus resinosa*, and E) the probability of feeding initiation by *Orthotomicus erosus* on filter papers treated with the solvents only. Comparison between these means is not appropriate, but the effect of the extracted chemicals is described by the comparison of parameters for the extracts and the solvent controls. A higher than expected value denotes feeding incitant activity, whereas a lower value denotes feeding suppressant activity.





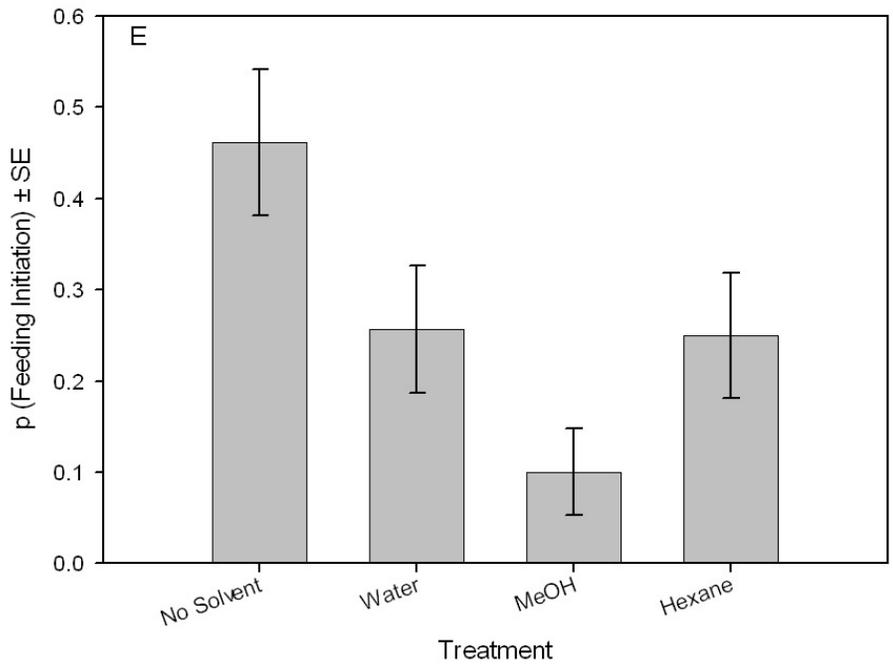
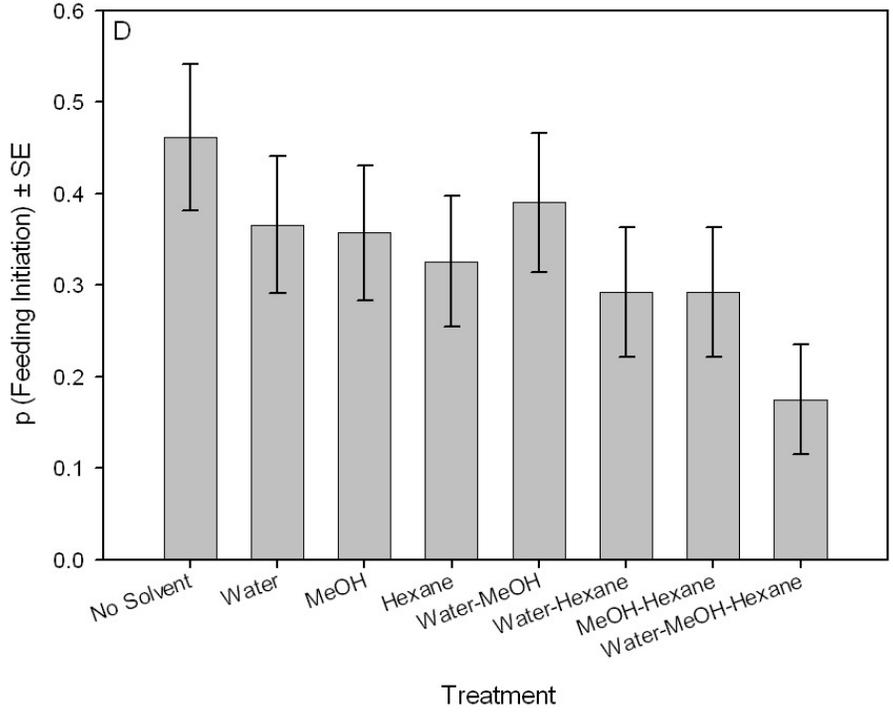
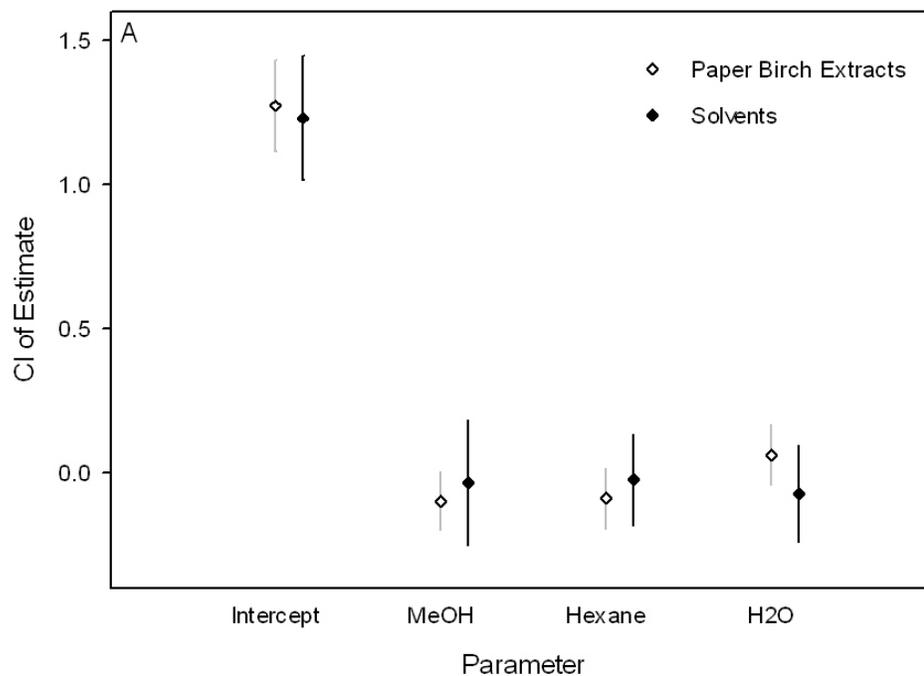
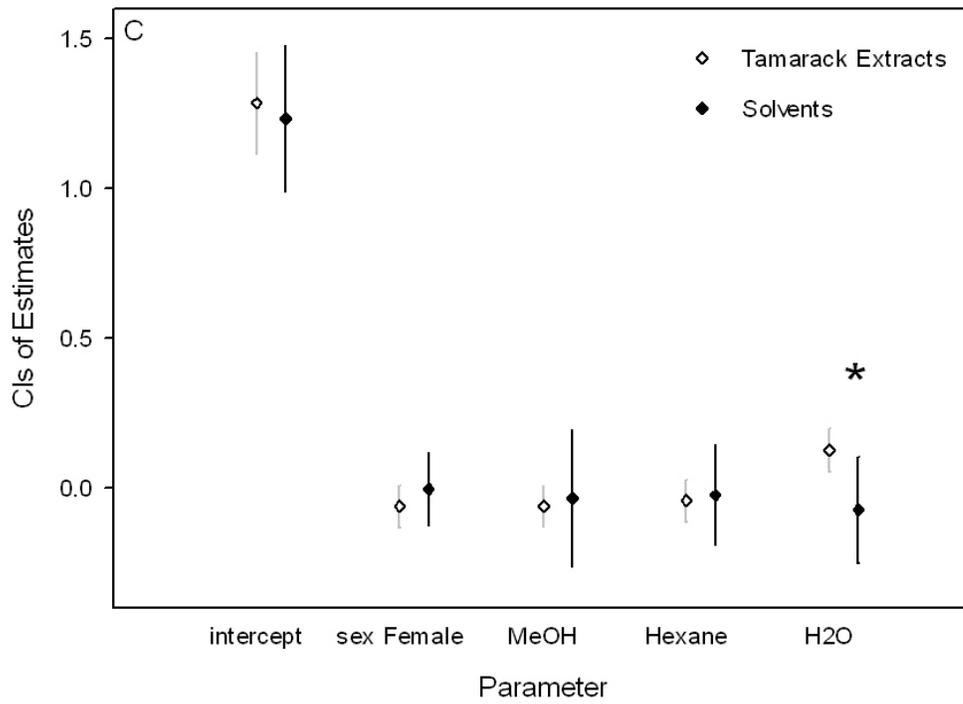
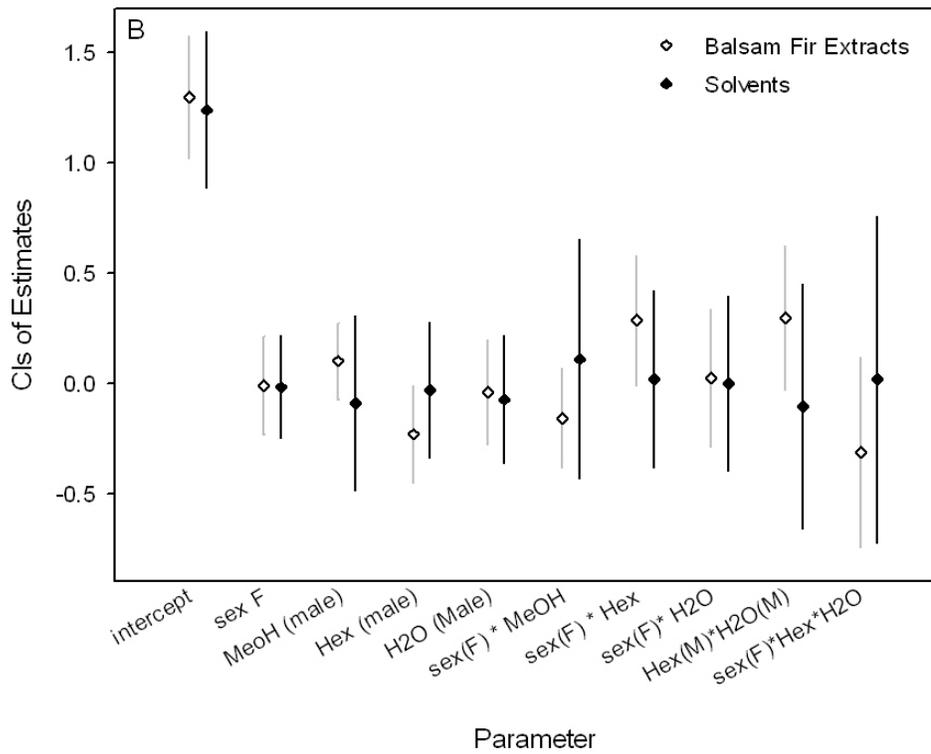


Fig. 4.3. Simultaneous 95% profile likelihood confidence intervals (CIs) of parameter estimates for each species from the logistic regression models of the effect of each extract from A) *Betula papyrifera*, B) *Abies balsamea*, C) *Larix laricina*, and D) *Pinus resinosa* and the corresponding solvents on the amount of feeding by *Orthotomicus erosus* that initiated feeding. Estimates for parameters associated with beetle sex or solvent interactions were estimated only when those terms met criteria for inclusion in the model. Cases where the difference of the parameter value of the extracts and the corresponding solvent does not overlap with zero are marked with a *. A higher value of response elicited by the extract than by the blank denotes feeding stimulant activity, whereas a lower value denotes feeding deterrent activity.





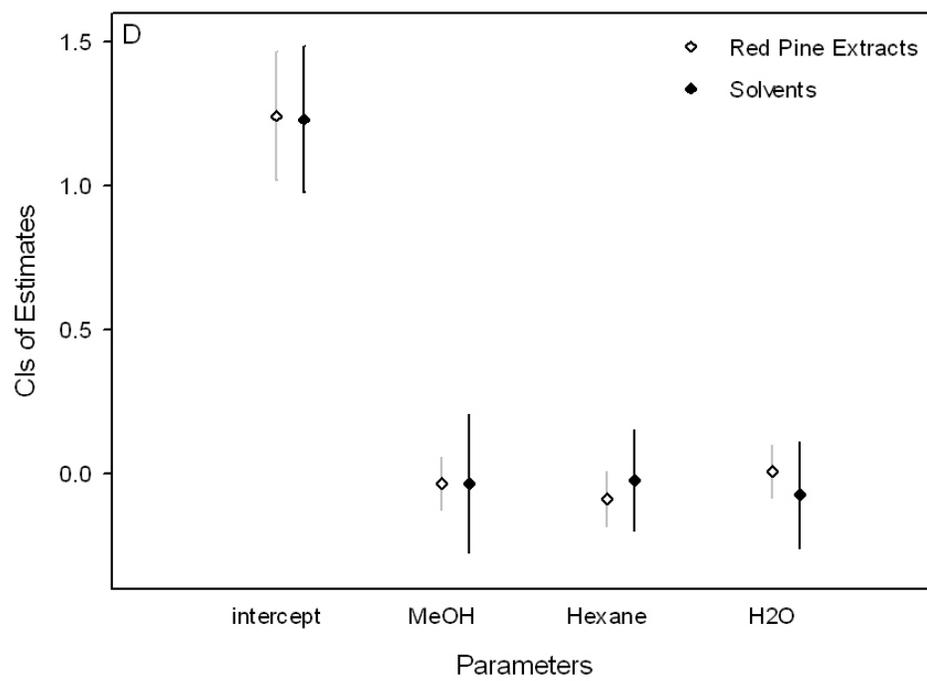
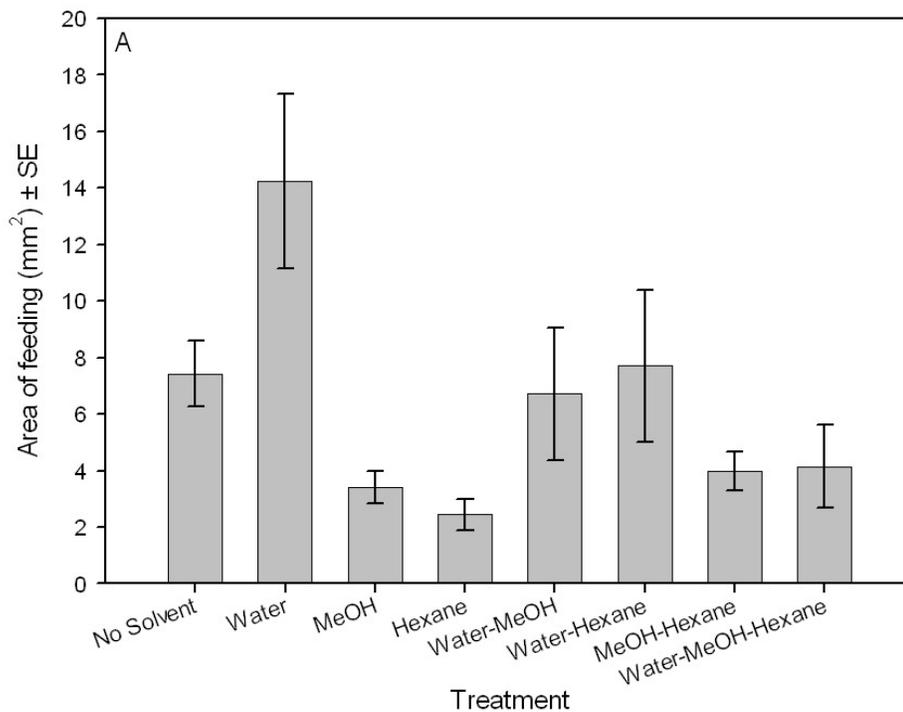
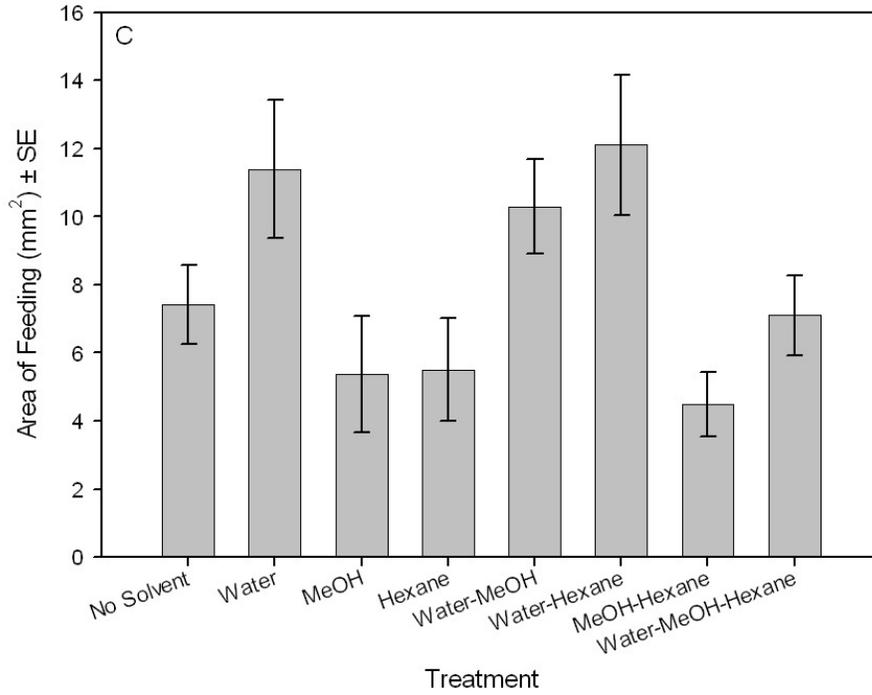
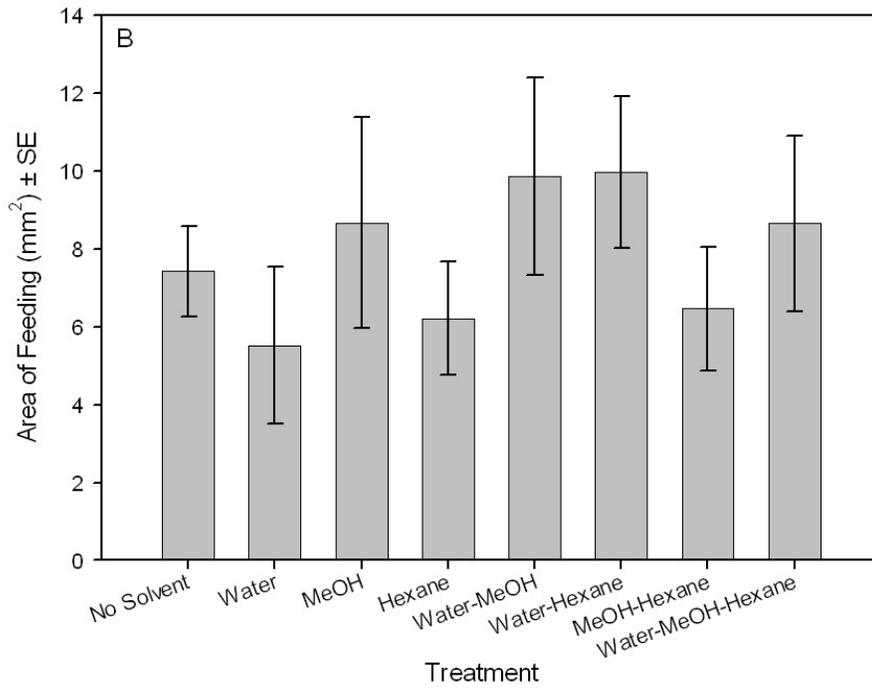
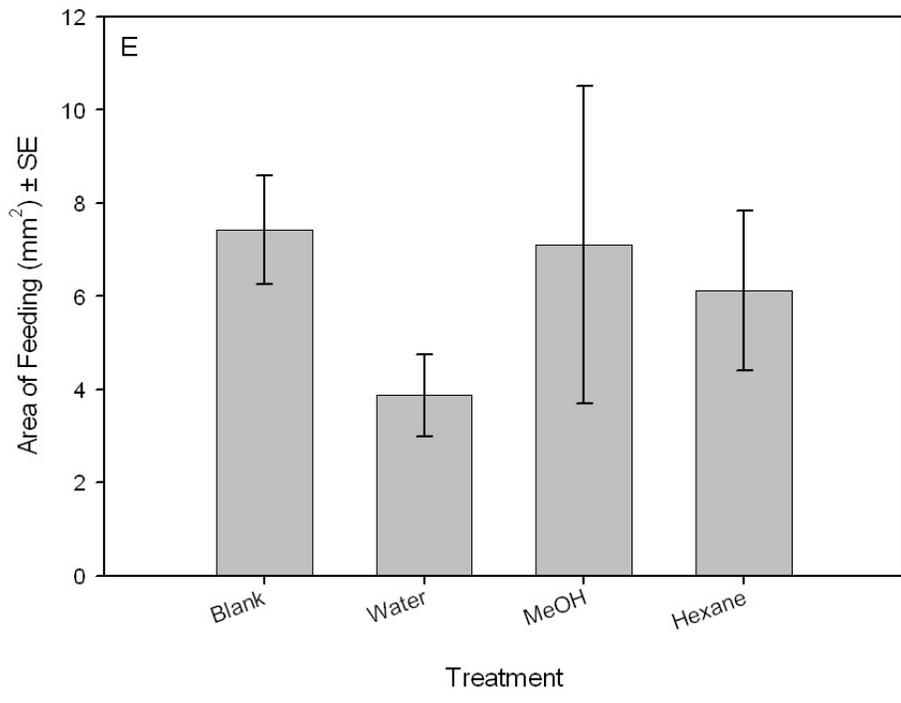
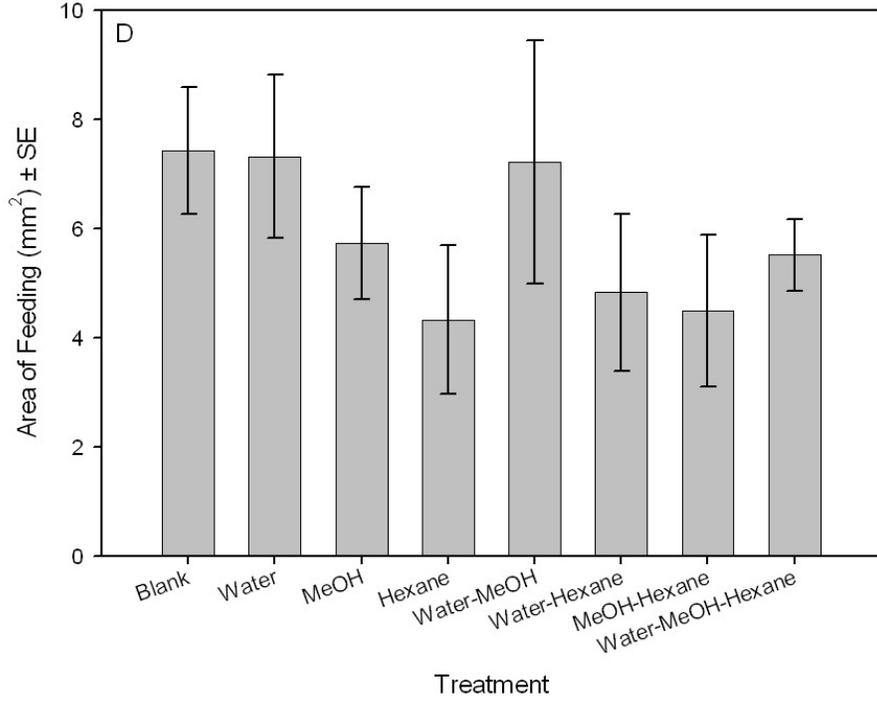


Fig. 4.4. Mean and standard error of the extent of feeding on filter paper containing single and combined extracts of A) *Betula papyrifera*, B) *Abies balsamea*, C) *Larix laricina*, and D) *Pinus resinosa*, and E) the extent of feeding on filter papers treated with the solvents only. Only cases where feeding was initiated are included in the presentation. Comparison between these means is not appropriate, but the effect of the extracted chemicals is described by the comparison of parameters for the extracts and the solvent controls. A higher than expected value denotes feeding stimulant activity, whereas a lower value denotes feeding deterrent activity.







Chapter 5. Plant phylogeny is a poor determinant of host range for an oligophagous insect invader

Abstract To anticipate and manage the consequences of geographic range expansion by invasive herbivorous insects, it is necessary to predict host use in association with novel plant species. The relatedness between a known host or non-host plant and a plant of interest is often used to forecast the response of an insect herbivore to the plant of unknown host status. Here, I examine the relationship between the genetic relatedness of a set of plants and two components of host use: acceptance behavior and reproductive potential. The study insect is *Orthotomicus erosus* (Wollaston), an oligophagous bark beetle poised to invade much of North America; the plants are North American tree species from Pinaceae and Cupressaceae. We also test the ability of significant regressions of beetle response against plant phylogenetic distance to predict data from independent validation datasets. Bark boring, a component of adult host selection was not related to plant phylogeny and could not be predicted from it. In contrast, reproductive potential was related to phylogenetic distance between plant species, but the information we used was not sufficient to make precise predictions. For oligophagous insects such as *O. erosus*, the phylogenetic relatedness between potential hosts may not be an appropriate predictor of host range

Keywords: Scolytidae, bark beetle, Mediterranean pine engraver, Pinaceae, phylogeny, novel host plant, insect-plant interactions, herbivore host range, biological invasion, prediction, genetic distance, random permutation test, logistic regression

Introduction

The geographic ranges of many herbivorous insect species are undergoing rapid expansion due to factors such as global climate change, inadvertent introductions associated with global trade, and deliberate introduction for the purposes of agriculture, horticulture, or classical biological control. As species invade new areas, novel plant-herbivore associations are likely. Range expansion may have ecological and evolutionary consequences. Prediction of host use by herbivores in association with novel plant species is necessary so that negative impacts can be prevented or mitigated. The genetic relatedness between a known host or non-host plant and a plant of interest is often used to forecast the response of an insect to the plant of unknown host status (i.e., Wapshere 1974). However, the relationship between plant phylogeny and insect host range is unclear, especially for oligophagous or polyphagous insect species.

The ability of an herbivorous insect to reproduce on a particular plant and insect's decision to utilize the plant are governed by the plant's traits (chemical, morphological, and ecological), and the insect's perception and response to those traits. If the relevant traits of the plant and the responses of the insect are known, then host use can be predicted directly. However, if any of the characteristics are unknown, then the results of the association must be predicted from other factors. If members of a population of the insect species use the same plant traits for host selection and the (unknown) plant traits are heritable, then the (known) phylogenetic relatedness between the novel plant and known host and non-host plants of the insect may be a useful predictor of insect host use on novel species (Symons and Beccaloni 1999).

Plant phylogeny has been used to predict insect host range in a number of cases. Closely related plants often share a more similar insect fauna than more distant relatives (Odegaard et al. 2005, Brandle and Brandl 2006, Gobner et al. 2009). Certain groups of specialized insect species, such as bruchine beetles (Coleoptera: Bruchidae), have closely related host plants (Kergoat et al. 2007). Plant phylogeny is used to inform the selection of plant species tested during host-range testing of highly specialized insects for use as weed biocontrol agents (Wapshere 1974, Briese and Walker 2002). Many types of insects colonize novel hosts that are closely related to their historical host plants. This pattern occurs regardless of whether the insect (Erbilgin et al. 2007) or the plant (Sauvard 2004, Brockerhoff et al. 2006a, Roques et al. 2006) has been introduced, although the pattern is stronger for specialist insects than for generalists colonizing introduced plants (Gobner et al. 2009).

In the same manner that insect host use should be predictable from phylogeny if host use relies on heritable plant traits, host use will not be predictable if the relevant plant traits are not correlated with plant relatedness (Jaenike 1990, Mitter and Farrell 1991, Agosta 2006, Brooks et al. 2006). For example, phenology of the host may govern interaction with an herbivore, but phenology may be more correlated to the environment than to genetics. Secondary compound chemistry (Becerra 1997) and plant defensive strategy (Agrawal and Fishbein 2006) within closely related groups of plants are not associated with phylogeny, but some secondary compounds that are important to insect host selection, such as glucosinolates, are found in several phylogenetically divergent groups (Schoonhoven et al. 2005).

The prediction of insect host range is especially important when an insect is the novel to the ecosystem. Upon the arrival of a novel herbivore to an ecosystem, there will be strong selection on the insect to utilize novel plants (Payne et al. 2004), regardless of the relatedness of the novel plants to the traditional hosts of the insect. Declining acceptance thresholds resulting from the physiological state of the insect (i.e., starvation or egg load, Courtney et al. 1989) make introduced herbivores more likely to attempt novel hosts. However, the reaction of oligophagous and polyphagous insect species to novel hosts in the absence of their normal hosts has seldom been explicitly studied (but see Ernst et al. 2007). Assuming that the host used by the insect in its native environment is rare or absent in the new environment and that many insects will attempt to feed on a novel host plant rather than starve, the choice of a novel host by an insect in a new environment may depend as much on the abundance and distribution of plant species in the new habitat as the relatedness of the historical hosts and novel plants.

Plants possess two non-exclusive sets of traits that are important for host use by insects. First, there is a set of traits that determine whether a plant is selected for feeding or oviposition: the acceptability of the plant (Schoonhoven et al. 2005). Second, there is a set of traits that determine the reproductive potential of an insect utilizing that plant: the suitability of the plant. For insects with immobile larvae, the adults evaluate the acceptability of the plant, but the larvae must deal with the effects of consuming the plant. The set of acceptable plant species and the set of suitable plant species for an insect may not be identical. A number of cases are known where the larvae of an insect are able to develop on plants that are not acceptable to adult insects (i.e., Wiklund 1975, Roininen and Tahvanainen 1989, Gratton and Welter 1998, Janz et al. 2001, Sauvard

2004). There are also known instances of introduced plants that are acceptable to adults but not suitable to the larvae of an insect species (Straatman 1962, Chew 1977, Copp and Davenport 1978, Rodman and Chew 1980, Harris et al. 2001, Barre et al. 2002, Graves and Shapiro 2003, Badenes-Perez et al. 2006, Casagrande and Dacey 2007). Thus, both plant acceptability and plant suitability must be understood in order to predict host use by insects.

The goal of this analysis was to explore the relationship between plant acceptability and suitability to the phylogenetic relatedness of plants for an oligophagous insect herbivore, an exotic invasive bark beetle. I hypothesized that this beetle species' reproductive potential and adult acceptance behavior on different plant species should be related to the phylogenetic distance among plants, and that the relationship between beetle response and phylogenetic distance from known host or non-host plants can be used to predict the response of the insect to novel species. I modeled the relationship between the phylogenetic distance between potential host tree species and two components of host use by the beetle: reproductive potential and host acceptance. I tested predictions from our significant models with independent validation datasets.

Methods and Materials

Study system. *Orthotomicus erosus* (Wollaston) is a bark beetle native to the Mediterranean and Asia that has been recently detected in California (Lee et al. 2008). Thus far, the beetle is apparently confined to ten counties, but future spread across North American range seems possible (Seybold and Downing 2009). Much of the concern about *O. erosus* stems from its potentially large host range. The beetle appears to feed on many *Pinus* spp. and has been associated with several other hosts from the families Pinaceae and Cupressaceae in its native and adventive geographic range (Mendel and Halperin 1982, Wood and Bright 1992, Bright and Skidmore 1997, Lee et al. 2008).

Several components of host use by *O. erosus* on North American tree species are known. Testing of larval developmental ability and adult acceptance behavior on North American tree species has occurred in the adventive range in California, USA and under quarantine condition in St. Paul, MN, USA (USDA APHIS Plant Protection and Quarantine Division Permit 74447) (Lee et al. 2008, Walter et al. in review-a, Walter et al. in review-b). In these experiments, several North American conifer species in the genera *Pinus*, *Picea*, and *Pseudotsuga* supported at least enough reproduction by the beetle to replace the parent generation; these species were considered suitable. Species from a larger set of genera, including unsuitable members of the genera *Abies* and *Tsuga*, were accepted by adult beetles when in contact with the outer bark. Host acceptance by bark beetles may consist of behaviors other than bark boring, for example landing rates may differ between tree species or galleries could be abandoned after the beetle begins boring. However, the behavior of *O. erosus* was not affected by bark and phloem odors of potential host trees (Walter et al. in review-b) and did not differ between tree species

after boring was initiated (Walter et al. in review-a). Therefore, I considered bark boring behavior to be indicative of the entire process of host acceptance for *O. erosus*. I analyzed the relationship between plant phylogeny and two components of host use by the beetles: suitability for development and acceptability to adults.

Plant phylogeny and phylogenetic distance. To estimate the phylogenetic distance between plant species, the sequences of the chloroplast gene *matk*, the mitochondrial gene *nad5*, and the nuclear gene 4CL used by Wang et al. (2000) were supplemented with more recently available data, including several new outgroups (*Gnetum gnemon*, *Metasequoia glyptostroboides*, *Podocarpus macrophyllus*, *Taxus canadensis*) (Table 5.1). Sequences for the 4CL gene were not available for the outgroups. However, missing data in some groups does not necessarily decrease the accuracy of the phylogenetic analysis as long as the data that is present is sufficient to distinguish groups from each other (Kearney and Clark 2003, Wiens 2003). In this case, the outgroup could be unambiguously separated from the ingroup on the basis of only the two included genes (see results). The sequences were aligned with Clustal W (Thompson et al. 1994), and the alignment was revised manually. After alignment, we identified an 890 base pair region of *matk*, an 1122 base pair region of *nad5*, and a 759 base pair region of 4CL to use in the phylogenetic analysis. Models of nucleotide substitution were selected using MrModeltest version 2.3 (<http://www.abc.se/~nylander/>, Nylander 2004). The GTR+G model was implemented for *matk*, the SYM+G model was implemented for *nad5*, and the K80+G model was implemented for 4CL. The partitioned dataset was analyzed via Markov chain Monte Carlo analysis with MrBayes version 3.1.2 (mrbayes.csit.fsu.edu,

Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). MrBayes was run for 1,000,000 generations, with the first 25% used as burn-in data (excluded from further analysis because the likelihood had not yet plateaued). Trees were displayed in Treeview version 1.6.6 (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>, Page 1996).

Phylogenetic distances were estimated as the sum of branch lengths between taxa from the consensus tree generated in MrBayes. Phylogenetic distance was used instead of traditional genetic distance to obtain a combined distance measure from the three genes in our dataset and to provide information about the hierarchical nature of the relationship between species. Phylogenetic distances were calculated from the basal node of each genus because not all of the species used in our laboratory experiments were included in the phylogenetic analysis. If there was only one member of a genus in the dataset, then the phylogenetic distance was calculated from that tip. The branch lengths from the basal nodes of genera to the species tips in the tree were very small (branch lengths of 6×10^{-3} - 48×10^{-3}); if species not included in the phylogenetic analysis have similar values, calculating phylogenetic distance from the base of a genus is unlikely to affect the results. For species within the Cupressaceae, distances were calculated from the congeneric genus *Metasequoia*, because that genus was the only representative of Cupressaceae in the outgroup. For purposes of comparison, mean character distances were calculated using PAUP* (Swofford 2003).

Model building. Data on the reproductive potential and bark boring behavior of *O. erosus* on red pine, *Pinus resinosa*; white spruce, *Picea glauca*; tamarack, *Larix laricina*; eastern hemlock, *Tsuga canadensis*; and balsam fir, *Abies balsamea*, taken from

previously described experiments (Table 5.2) (Walter et al. in review-a, Walter et al. in review-b) were regressed against the phylogenetic distance from the tree genus to other tree genera in our dataset. Each response was coded as a binomial variable.

Reproductive potential was coded according to whether a set of fifteen adult beetles produced enough viable offspring to replace the parents when forced to colonize logs of five North American conifer species. Bark boring behaviors were coded based on whether individual beetles bored into the outer bark of potential hosts under no-choice conditions.

Bark boring behavior and net reproduction were analyzed in several separate logistic regression models (Table 5.3). Red pine was considered the good host because beetles introduced into red pine reproduced enough to replace themselves in >50% of the replicates. White spruce was considered a suboptimal host because beetles were able to replace themselves 0-50% of the time. Of the three non-host species (tamarack, eastern hemlock, and balsam fir), tamarack was most closely and eastern hemlock was most distantly related to the hosts. These models were selected because we felt that the combinations represented (one tree suitable for reproduction, a marginally suitable tree, and three unsuitable trees at various distances) would allow a qualitative estimation of the type of knowledge about insect host use needed to make accurate predictions.

My logistic regression models were constructed as follows: the number of positive responses, y , in a group vector \mathbf{x} was divided by the number of trials in each group, \mathbf{n} , to generate a probability $\theta(\mathbf{x})$ of a positive response, which could take any value between 0 and 1. To linearize the probabilities, a logit transformation was carried out to generate g following the equation

$$g = \log\left(\frac{\theta(\mathbf{x})}{1 - \theta(\mathbf{x})}\right) \quad (1)$$

A linear relationship between the logit and the independent variable(s) can then be predicted with the form

$$g = \boldsymbol{\beta}'\mathbf{x} \quad (2)$$

(Weisberg 2005) where $\boldsymbol{\beta}$ is the vector of regression parameters associated with the independent variables \mathbf{x} . Logistic regression was performed with the SAS software package version 9.1 (PROC LOGISTIC, SAS Institute Inc. 2004). Our regressions of the full (5 distance) models were fully specified and did not include an intercept, but all submodels did include an intercept so that we could predict the beetle response to species congeneric with the predictors. We did not use the significance test provided by the software to evaluate the model because the assumptions of the test were violated.

Model significance. The five tree species that I tested are evolutionarily related to each other. Thus, the genetic distance from one species to the others cannot be considered independent data points (two species that are both far away from a reference species are likely to be more closely related than a species near to a reference point and a species far from it) (Felsenstein 1985). The regression models that I generated cannot be tested against a known distribution (such as a χ^2 or an F statistic) because of this violation of assumptions (Felsenstein 1985, Manly 2007). Instead, I considered a model to be significant if the likelihood was obtained was unlikely to be obtained by randomly reordering the response data. We randomly reordered the response data 10,000 times, and performed a separate regression each time to generate a null distribution of the

possible likelihoods that could have arisen from our data. The significance of the (not re-ordered) data was then obtained by calculating the proportion of the null distribution with a higher likelihood than the original data (a random permutation test, Manly 2007). The significance of the individual terms was calculated in the same way. A one-sided test was used to evaluate the parameters; negative parameters were evaluated with a left-sided test and positive parameters with a right-sided test. Permutations of the data were carried out with the %rand_gen macro for SAS (Cassell 2002). The permutations for each submodel that we evaluated were carried out with a different random seed, taken from a random number table. The significance of each model was assessed with a Bonferroni-adjusted *P*-value for each response (*P*-value of 0.0042 for each model, experimentwise *P*-value of 0.05 for all models of larval development or adult bark boring).

Model validation. For the validation of the models of bark boring behavior, we conducted a laboratory experiment using jack pine, *Pinus banksiana*; red pine, *Pinus resinosa*; black spruce, *Picea mariana* from Grand Rapids, MN; and northern white cedar, *Thuja occidentalis* from near Warba, MN. The methods were the same as previously described (Walter et al. in review-a), and used ca. 56 male and 56 female beetles for each tree species (total beetles = 443).

For the validation of the models of reproductive potential, we used data from a previously published experiment where *O. erosus* was forced to colonize various species and net reproduction was reported (Lee et al. 2008). The data were recoded to reflect whether the nine beetles introduced into each replicate log reproduced enough to replace the parents. In addition, we tested one species from the Cupressaceae, northern white

cedar, *Thuja occidentalis*, under quarantine conditions in St. Paul, MN using the same methods as for the prediction dataset because this species was included in our behavioral datasets. Standard error for the probability that parent beetles introduced into a log would replace themselves were calculated using the incomplete beta distribution (Kvanli 1988) because *O. erosus* frequently achieved replacement rate for either all or none of the five to seven replicates for a given species.

Two approximations were necessary to include species from Cupressaceae in our validation data. The branch lengths from members of the Pinaceae to the members of the Cupressaceae in the validation datasets (redwood and northern white cedar for development and northern white cedar for the behaviors) are underestimated because of the missing data for the 4CL gene in the outgroup taxa. These underestimated branch lengths are much longer than any that were used in the prediction dataset, so predictions for members of the Cupressaceae represent a large extrapolation of the original model. The phylogenetic distance to any member of the family Cupressaceae was approximated as the phylogenetic distance to the genus *Metasequoia*, because that genus was included in the phylogenetic analysis. Other experiments studying the relationship between host range and host phylogeny have used genetic data from close relatives when sequences for the species of interest were not available (Odegaard et al. 2005, Brandle and Brandl 2006)

We evaluated the predictive ability of the significant models by comparing the confidence intervals of the predictions obtained from the models to the observed responses in the validation experiments. Predicted probabilities and simultaneous 95% confidence intervals of prediction were obtained for each species in the validation

datasets for each of the significant models for bark boring behavior and net reproduction (PROC LOGISTIC, SAS Institute Inc. 2004). If this prediction interval did not include the probability of success (reproduction at or above replacement rate or boring into the outer bark) from the validation dataset, the submodel was unable to predict the observed response in the validation dataset.

Effect of missing distances. The sequences available for phylogenetic analysis did not include all of the species used in the experiments with beetles. Therefore, I calculated phylogenetic distance between the basal nodes of genera rather than between species, and our phylogenetic distances may be underestimated. To examine the possible effects of mis-estimation of phylogenetic distances, I examined the mean character differences between species in our predictive model and species used in the phylogenetic model where sequences were available, and repeated the logistic regression after manipulating the phylogenetic distance matrix in three ways: I increased or decreased the phylogenetic distance from *Pinus* to the other genera by 5% and replaced the phylogenetic distance from the genus *Larix* with the phylogenetic distance from *Larix laricina* to the basal nodes of the other genera. Logistic regression for each of the three data manipulations was performed as described above, and the likelihood of the models was evaluated with a random permutation test. For comparison purposes, genetic distance based on mean character difference was calculated for *nad5* and *matk* when sequences for species used in the predictive experiment were available using Paup* (Swofford 2003).

Results

Plant phylogeny and phylogenetic distance. The unrooted tree recovered in our analysis is equivalent to the tree recovered by Wang et al. (2000) (Figure 5.1). Our analysis differs from the previous work by incorporating several additional outgroups (*Gnetum gnemon*, *Metasequoia glyptostroboides*, *Podocarpus macrophyllus*, *Taxus canadensis*). Phylogenetic distance (the sum of the branch lengths between the basal nodes of genera, Table 5.4) was different from genetic distances obtained from mean character differences (Table 5.5).

Relationship between beetle response and plant phylogeny. We generated a total of 24 models describing the relationship between either net reproduction or bark boring behavior of *O. erosus* in novel conifer species and the phylogenetic distance between plant genera (Table 5.6 and 5.7). The tamarack-only model was significant for adult bark boring behavior, but all models except the spruce-only model were significant for larval development. For bark boring, tamarack was the species with the lowest boring response. Reproductive potential of *O. erosus* on novel tree species is correlated with the phylogeny of the trees, but acceptance of the same tree species by the beetle is not correlated with plant phylogeny.

Model validation. The significant models of host acceptance were validated with the results of an independent laboratory experiment that used methods identical to the original experiment (Table 5.8). The tamarack only model was able to predict the bark

boring response of beetles on red pine (Fig. 5.10), which was included in the prediction and validation datasets. The response of *O. erosus* other species in the validation experiment was not predicted from this model.

Models of net reproduction developed from one set of laboratory experiments were validated from the results of an independent experiment (Table 5.8). Most of the eleven significant models of net reproduction predicted beetle development on *Pinus monophylla*, *Larix laricina*, and *Abies concolor* (Table 5.9). Reproduction on *Picea glauca* was predicted by four of the eleven models. When the prediction was accurate, the range of the prediction interval was very large (i.e., a range < 75%). In some cases, the prediction interval encompassed all possible probabilities, and it was only in these cases that any of the models predicted reproduction on *Pseudotsuga menziesii*, *Thuja occidentalis*, or *Sequoia sempervirens*. More precise knowledge about branch lengths would probably not improve the accuracy of predictions about species from the Cupressaceae because the predictions are extrapolated far beyond the bounds of the prediction dataset. Although there is a statistically significant relationship between plant phylogeny and beetle reproductive potential, our models were not able to generate precise for any of the tree species or accurate predictions for most species.

Effect of missing distances. The mean character differences between species in our predictive laboratory experiment and species in the phylogenetic analysis were small, showing that our models were probably not affected substantially by calculations from the basal node of a genus rather than from individual species or by the use of different species in the phylogenetic analysis and experiments with the beetles (Table 5.11). The

models of net reproduction calculated using altered phylogenetic distances to *Pinus* or phylogenetic distances from *Larix laricina* (Table 5.12) did not change in significance due to the manipulations of the data. The significance of the models of adult acceptance also did not change except that the tamarack-only model became insignificant and the red pine + tamarack model became significant when phylogenetic distances were calculated from *Larix laricina* instead of from the base of *Larix* (Table 5.13). The *P*-value calculated from these models was very near the significance threshold and changed by 0.0005 and 0.0008 respectively because of our data manipulation. I do not consider this to be a meaningful change.

Discussion

The host acceptance behavior of *O. erosus* does not appear to be related to plant phylogeny. The exception to this trend was the model containing the distance to tamarack, which had the lowest bark boring response. The significant predicted the acceptance of red pine, a species included in the prediction and validation experiments, but not the response to any other species. This is important because it shows that the remaining species were not predicted because of differences between species, and not due to differences between the prediction and validation experiments.

In contrast, the net reproduction of *O. erosus* on novel tree species is more related to the phylogeny of the trees than could be expected by chance, but knowledge of plant phylogeny does not yield very precise or accurate predictions of beetle reproduction. All but one of the models of *O. erosus* reproduction were significant. The exception was the model based solely on the phylogenetic distance to white spruce. That model was probably not significant because white spruce, a marginally significant species, is approximately equidistant from a suitable species and unsuitable species. When the predictions from our significant models compared to the reproductive potential on *O. erosus* from an independent experiment, the models predicted the reproduction potential on several species, although the predictive intervals were large.

Based on the results of the analysis, I conclude that *host suitability* of novel tree species for *O. erosus* will be related to the phylogenetic relationships between the novel plant species encountered by the insect and known hosts or non-hosts. However, adult *acceptance* of novel tree species will not. Introduced populations of the beetles may

distribute their eggs among a set of plants in the novel environment that is different from the set of plants where they would be capable of reproducing. If the accepted set of plants is broader than the set of suitable plants, accepted but unsuitable plants may act as a reproductive sink. If the accepted set of plants is narrower than the set of suitable plants, the beetles may under-utilize available resources. Either situation could have negative consequences at the population level, and could affect the ability of the beetle to establish in a new area.

The results of this project differ from assumptions about the importance of host phylogeny for insect host range used in the design of methods of biological control safety testing (Wapshere 1974, Briese and Walker 2002). However, there are several important differences between safety testing and the experiments reported here. First, safety testing is designed to confirm that a species already considered highly specialized based on historical literature and field observations is indeed a specialist. The experiments reported here were carried out on an oligophagous insect known to consume species from several genera within Pinaceae, and possibly also from other families as well (Mendel and Halperin 1982, Wood and Bright 1992, Bright and Skidmore 1997, Lee et al. 2008). Secondly, safety testing for biological control is designed to give a binary (yes/no) answer to the question of whether an insect is highly specialized, not to delimit the entire host range. For *O. erosus*, we already knew that the insect was not a specialist, but we were interested in describing the potential North American host range of the insect. Host range safety testing for biological control often uses the centrifugal phylogenetic method, a method where a series of potential hosts at greater and greater phylogenetic distance from a known host are tested. The designer of the method (Wapshere 1974)

acknowledged that the method will fail if host choice is based on a lack of deterrent stimuli, or if hosts occur in several distantly related clades based on the presence of a common stimulant. Both of these situations are likely in the case of oligophagous or polyphagous insects such as *O. erosus*.

Prediction of insect host use from plant phylogeny is difficult for several reasons. Plant traits important to insects and their possible relationships with plant phylogeny may be unknown. In this test I assumed that a phylogenetic dataset of sufficient size to describe the hierarchical relationships among taxa will also contain an accurate measure of the overall similarity between the taxa. However, if smaller datasets are used, the accepted phylogeny may not be recovered, and the distances between species may not be representative of their similarity. Finally, phylogenetically-derived data may not be suitable for some frequently-used statistical methods. For example, taxa sampled from a branching phylogeny are not independent (Felsenstein 1985), so probability based statistics such as the F test are not appropriate. This final complication can be circumvented by a random permutation test (Manly 2007).

Several systems are known where insect host use is not well-correlated with plant phylogeny. There are examples from Coleoptera, including Chrysomelid beetles in Papua New Guinea (Pokon et al. 2005) and within the new-world genus *Blepharida* (Becerra 1997), and seed-feeding Bruchid beetles in Africa (Kergoat et al. 2005); from Diptera, a leaf-mining fly *Liriomyza helianthi* (Gratton and Welter 1998); and from Lepidoptera, Papilionidae (Miller 1987, Thompson et al. 1990). Lack of correlation between phylogeny and host use may arise because the stimulatory traits determining host use are phylogenetically disjunct. Another situation where insect host use may not

be associated with plant taxonomy occurs where insect host selection is based on the absence of deterrent traits, rather than the presence of stimulants. Deterrents will be encountered in most plants by most insects (Schoonhoven et al. 1998), and may be especially important in determining the host range of oligophagous and polyphagous species (Thorsteinson 1960, Jermy 1966, Renwick 2001).

Biological invasions are among the most important sources of change challenging ecosystems today (Vitousek et al. 1997, Mack et al. 2000). Accurate about potentially invasive insects, including their potential host range, are necessary so that limited management resources may be properly deployed. I found significant relationships between beetle reproductive ability and phylogenetic distance. However, the relationship between beetle host acceptance behavior and phylogenetic distance was not different than should be expected by chance. Furthermore, the models of reproductive ability and host acceptance behavior were not able to forecast the response of the beetle to a different set of tree species examined in independent experiments. For oligophagous insects such as *O. erosus*, host plant phylogeny appears to be a poor predictor of host range.

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Table 5.1 GenBank sequences used to re-construct the phylogeny of the Pinaceae.

Species	<i>nad5</i>	<i>matk</i>	4CL
<i>Abies firma</i> Sieb. et Zucc.	AF143419	AF143436	AF144514
<i>Cathaya argyrophylla</i> Chun et Kuang	AF143415	AF143435	AF144505
<i>Cedrus atlantica</i> Manetti	AF143424	AF143431	AF144529
<i>Keteleeria evelyniana</i> Mast.	AF143418	AF143430	AF144522
<i>Larix kaempferi</i> (Lam.) Carrière	DQ087903	AF295028	AF470438
<i>Larix gmelinii</i> (Rupr.) Rupr.	AF143417	AF143433	AF144512
<i>Larix laricina</i> (Du Roi) Koch	DQ087901	AF295029	AF470450
<i>Larix potaninii</i> Batalin	DQ087910	AY389137	AF538062
<i>Nothotsuga longibracteata</i> Hu ex Page	AF143420	AF143437	AF144523
<i>Picea abies</i> (L.) Karst.	DQ358169	AB019899	AM265585
<i>Picea sitchensis</i> (Bong.) Carrière	DQ358198	AY035203	AY634363
<i>Picea smithiana</i> (Wall.) Boiss	AF143414	AF143429	AF144504
<i>Pinus armandii</i> Franch.	AF143413	AF143428	AF144501
<i>Pseudolarix amabilis</i> (Nelson) Rehd.	AF143423	AF143432	AF144527
<i>Pseudotsuga menziesii</i> (Mirbel) Franco	AF143416	AF143439	AF144506
<i>Tsuga canadensis</i> Carr.	AF143422	AF143438	AF144525
<i>Tsuga mertensiana</i> (Bong.) Rydb.	AF143421	AF143434	AF144524
<i>Cycas revoluta</i> Thunb.	AJ130743	AB116583	-
<i>Gnetum gnemon</i> L.	AJ409110	AF542561	-
<i>Metasequoia glyptostroboides</i> Hu & Cheng	DQ406973	AF152203	-
<i>Podocarpus macrophyllus</i> (Thunb.) Sweet	DQ406963	AF228111	-
<i>Taxus canadensis</i> Marsh.	AY159860	EF660661	-

Table 5.2 Prediction data used to evaluate the significance of the correlation between phylogenetic distance and beetle response and to build logistic regression models. Data on population replacement and probability of bark boring comes from previously reported studies.

Species	Population Replacement ¹		Bark Boring ¹	
	Prob.	N	Prob.	N
<i>Pinus resinosa</i> Aiton	0.7	10	0.48	97
<i>Picea glauca</i> (Moench) Voss	0.38	8	0.37	100
<i>Larix laricina</i>	0	8	0.26	100
<i>Tsuga canadensis</i>	0	8	0.35	202
<i>Abies balsamea</i> (L.) Mill.	0	8	0.36	102

¹ (Walter et al. in review-a)

Table 5.3 Rationale for the inclusion of different conifer species in models to predict the likelihood of *O. erosus* achieving population replacement or boring into the outer bark.

Model	Justification ¹	Species included
Full	Maximum information	<i>Pinus resinosa</i> , <i>Picea glauca</i> , <i>Larix laricina</i> , <i>Tsuga canadensis</i> , <i>Abies balsamea</i>
RP	Good host ² only	<i>Pinus resinosa</i>
WS	Suboptimal host ² only	<i>Picea glauca</i>
T	Nonhost only	<i>Larix laricina</i>
H	Nonhost only	<i>Tsuga canadensis</i>
BF	Nonhost only	<i>Abies balsamea</i>
RP + T	Good host + Near nonhost	<i>Pin. resinosa</i> , <i>L. laricina</i>
RP + H	Good host + Distant nonhost	<i>Pin. resinosa</i> , <i>T. canadensis</i>
WS + T	Suboptimal host + Near nonhost	<i>Pic. glauca</i> , <i>L. laricina</i>
WS + H	Suboptimal host + Distant nonhost	<i>Pic. glauca</i> , <i>T. canadensis</i>
RP + WS	Hosts	<i>Pin. resinosa</i> , <i>Pic. glauca</i>
T + H + BF	Nonhosts	<i>L. laricina</i> , <i>T. canadensis</i> , <i>A. balsamea</i>

¹ The submodels were chosen because I felt they were representative of possible states of knowledge about the host range of an insect prior to extensive host-range testing.

² *O. erosus* produced enough viable offspring to replace the parents when forced to colonize a species >50% of the time for a good host and <50% of the time for a suboptimal host.

Table 5.4 Phylogenetic distance (sum of branch lengths) between tree genera in the prediction dataset and genera in the prediction and validation datasets from a Bayesian analysis.

Genus ¹	Distance from <i>Pinus</i>	Distance from <i>Picea</i>	Distance from <i>Larix</i>	Distance from <i>Tsuga</i>	Distance from <i>Abies</i>
<i>Pinus</i>	0	0.115487	0.140109	0.163287	0.155289
<i>Picea</i>	0.115487	0	0.090464	0.102431	0.105644
<i>Larix</i>	0.140109	0.090464	0	0.10855	0.100552
<i>Pseudotsuga</i>	0.160499	0.110854	0.048864	0.12894	0.120942
<i>Tsuga</i>	0.163287	0.102431	0.10855	0	0.079688
<i>Abies</i>	0.155289	0.105644	0.100552	0.079688	0
<i>Metasequoia</i> ²	1.133994	1.084349	1.079257	1.058393	1.039315
<i>Thuja</i> ²	1.133994	1.084349	1.079257	1.058393	1.039315

¹ Host selection behaviors were validated using only representatives from *Pinus*, *Piceae*, and *Thuja*.

² Phylogenetic distances to genera within Cupressaceae are underestimated because of missing data from the 4CL nuclear gene and are all approximated as the distance to *Metasequoia*.

Table 5.5 Mean character distance (proportion of different base pairs) between genera used in the prediction and validation models. Where more than one species per genus was present in the phylogenetic analysis, distances are the average of each species in one genus to each species in the other.

	<i>Pinus</i>	<i>Picea</i>	<i>Larix</i>	<i>Psuedotsuga</i>	<i>Tsuga</i>	<i>Abies</i>	<i>Metasequoia</i>
<i>Pinus</i>	0	0.0767	0.1017	0.1108	0.0994	0.1020	0.5929
<i>Picea</i>		0	0.0633	0.0766	0.0685	0.0668	0.5976
<i>Larix</i>			0	0.0455	0.0767	0.0742	0.6025
<i>Psuedotsuga</i>				0	0.0826	0.0839	0.6082
<i>Tsuga</i>					0	0.0536	0.6024
<i>Abies</i>						0	0.5939
<i>Metasequoia</i>							0

Table 5.6 Logistic regression models and log(likelihood) generated for the probability that a group of beetles forced into a log would reproduce enough to replace themselves.

Model	Equation ¹	Ln(likelihood)	<i>P</i> ²
Full*	= -58.47 RP** + -75.99 WS** + 37.56T [†] + 14.16H [†] + 13.19BF [†]	-11.40	0.0004 [§]
RP*	= 1.07** + -25.09RP**	-14.34	<0.0001 [§]
WS	= -0.95 + -2.56WS	-23.01	0.7124
T*	= -6.99** + 53.06T**	-17.16	0.0006 [§]
H*	= -6.38** + 44.38H**	-14.79	0.0001 [§]
BF*	= -7.28** + 53.31BF**	-13.96	0.0001 [§]
RP+T*	= 0.22 + -22.40RP** + 6.16T	-14.27	0.0003 [§]
RP+H*	= -0.59 + -19.30RP [‡] + 10.02H	-14.26	0.0004 [§]
WS+T*	= -37.04 [‡] + -161.84WS** + 403.84T**	-11.40	0.0002 [§]
WS+H*	= -29.00** + -131.84WS** + 278.15H**	-11.40	<0.0001 [§]
RP+WS*	= 12.40** + -111.78RP** + -100.02WS**	-11.40	<0.0001 [§]
T+H+BF*	= -6.46 [‡] + 15.65T [†] + 13.63H [†] + 20.53BF [†]	-13.22	0.0003 [§]

¹ Slopes were calculated from the phylogenetic distance from the genera used in the model and the genera present in the prediction dataset. Parameters were: RP = phylogenetic distance from red pine, WS = phylogenetic distance from white spruce, T = phylogenetic distance from tamarack, H = phylogenetic distance from hemlock, and BF = phylogenetic distance from balsam fir. *P* values of the parameters were determined as the proportion of values generated from the randomly permuted datasets that were above (for positive values) or below (for negative values) the parameter calculated from the original data: † = 0.05 – 0.1, ‡ = 0.01 – 0.05, * = 0.005-0.01, ** > 0.005

² *P*-values are derived from a random permutation test. Significant models when the *P*-value for all 12 comparisons is held at 0.05 by the Bonferroni adjustment are marked with a [§].

Table 5.7 Logistic regression models and log(likelihood) generated for the probability that an individual beetle would bore into the outer bark of various tree species when confined under no-choice conditions.

Model ¹	Equation ¹	Ln(likelihood)	P ²
Full	= -4.75RP* + -0.84WS + 3.57T* + -1.20H + -1.73 BF	-388.17	0.0264
RP	= -0.11** + -3.74 RP*	-390.39	0.0105
WS	= -0.65 + 0.96WS	-393.54	0.6685
T	= -1.12** + 6.00T**	-389.32	0.0038 [§]
H	= -0.70 [†] + 1.85H [†]	-392.82	0.2007
BF	= -0.77 + 2.37BF	-392.82	0.2002
RP+T	= -0.70 + -2.37RP [†] + 4.62T [‡]	-388.21	0.0047
RP+H	= 0.46 [‡] + -6.40RP* + -3.23H**	-389.52	0.0154
WS+T	= -1.10 [†] + -0.30WS + 6.06T**	-389.31	0.0141
WS+H	= -0.83 + 1.38WS + 1.98H [†]	-392.62	0.3555
RP+WS	= -0.16 [†] + -3.71RP* + 0.54WS	-390.36	0.0398
T+H+BF	= -1.32** + 6.03T** + 1.88H + 0.66BF	-388.17	0.0126

¹ Slopes were calculated from the phylogenetic distance from the genera used in the

model and the genera present in the prediction dataset. Parameters were: RP =

phylogenetic distance from red pine, WS = phylogenetic distance from white spruce, T =

phylogenetic distance from tamarack, H = phylogenetic distance from hemlock, and BF =

phylogenetic distance from balsam fir. *P* values of the parameters were determined as

the proportion of values generated from the randomly permuted datasets that were above

(for positive values) or below (for negative values) the parameter calculated from the

original data: † = 0.05 – 0.1, ‡ = 0.01 – 0.05, * = 0.005-0.01, ** > 0.005

² *P*-values are derived from a random permutation test. Significant models when the *P*-

value for all 12 comparisons is held at 0.05 by the Bonferroni adjustment are marked with

a [§]

Table 5.8 Validation data for insect response used to evaluate the predictive abilities of logistic regressions between genetic distance and beetle response.

Species ¹	Population Replacement ²		Bark Boring ³	
	Prob.	N	Prob.	N
<i>Pinus resinosa</i>	1	4	0.5046	109
<i>Pinus banksiana</i> Lamb	1	4	0.6273	110
<i>Picea mariana</i> (Mill.)	1	4	0.6250	112
<i>Thuja occidentalis</i> L.	0 ²	6	0.4821	112
<i>Abies concolor</i> (Gond. & Glend.) Hildebr.	0	7	-	-
<i>Calocedrus decurrens</i> (Torr.) Florin	0	5	-	-
<i>Larix laricina</i>	0.5	4	-	-
<i>Picea glauca</i>	0.75	4	-	-
<i>Pinus canariensis</i> Smith	1	4	-	-
<i>Pinus contorta murrayana</i> (Balf.) Critch	1	7	-	-
<i>P. halepensis</i> Mill	1	4	-	-
<i>P. jeffreyi</i> Balf	1	7	-	-
<i>P. lambertiana</i> Dougl.	1	7	-	-
<i>P. monophylla</i> Torr. & Frem	0.8	5	-	-
<i>P. pinea</i> L.	1	4	-	-
<i>P. ponderosa</i> Dougl. ex Laws	1	7	-	-
<i>P. radiata</i> Don	1	5	-	-
<i>P. sabiniana</i> Dougl. ex Don	1	4	-	-
<i>P. strobus</i> L.	1	4	-	-
<i>P. sylvestris</i> L.	1	4	-	-
<i>P. taeda</i> L.	1	5	-	-
<i>Pseudotsuga menziesii</i> (Mirb.) Franco	1	5	-	-
<i>Sequoia sempervirens</i> Lamb	0	5	-	-

¹ Species are taken from Pinaceae except for *Sequoia sempervirens*, *Calocedrus*

decurrens, and *Thuja occidentalis* (all Cupressaceae).

² from (Lee et al. 2008)

³ data generated in separate validation experiments using methods from Walter et al. (in review-a)

Table 5.9 Rate of replacement of *Orthotomicus erosus* on tree species in the validation experiment and simultaneous 95% confidence intervals of the predicted rate of population replacement from the significant models. Species with the same number of replicates and rate of replacement are grouped. Cases where the prediction interval does not include the observed response are marked with a *.

Species	Rate of replacement (SE) ¹	Simultaneous 95% Confidence intervals of models										
		Full	RP	T	H	BF	RP + T	RP + H	WS + T	WS + H	RP + WS	T + H + BF
<i>Pinus resinosa</i> group ²	1.00 (0.16)	0.22, 0.95*	0.24, 0.96*	0.19, 0.91*	0.23, 0.95*	0.24, 0.96*	0.25, 0.96*	0.24, 0.96*	0.22, 0.95*	0.22, 0.95*	0.22, 0.95*	0.26, 0.97*
<i>Pinus jeffreyi</i> group ³	1.00 (0.11)	0.22, 0.95*	0.24, 0.96*	0.19, 0.91*	0.23, 0.95*	0.24, 0.96*	0.25, 0.96*	0.24, 0.96*	0.22, 0.95*	0.22, 0.95*	0.22, 0.95*	0.26, 0.97*
<i>Pinus radiata</i> group ⁴	1.00 (0.14)	0.22, 0.95*	0.24, 0.96*	0.19, 0.91*	0.23, 0.95*	0.24, 0.96*	0.25, 0.96*	0.24, 0.96*	0.22, 0.95*	0.22, 0.95*	0.22, 0.95*	0.26, 0.97*
<i>Pinus monophylla</i>	0.80 (0.18)	0.22, 0.95	0.24, 0.96	0.19, 0.91	0.23, 0.95	0.24, 0.96	0.25, 0.96	0.24, 0.96	0.22, 0.95	0.22, 0.95	0.22, 0.95	0.26, 0.97
<i>Picea glauca</i>	0.75 (0.22)	0.06, 0.85	0.03, 0.48*	0.01, 0.50*	0.02, 0.50*	0.03, 0.53*	0.03, 0.48*	0.03, 0.49*	0.06, 0.85	0.06, 0.85	0.06, 0.85	0.04, 0.56*
<i>Picea mariana</i>	1.00 (0.16)	0.06, 0.85*	0.03, 0.48*	0.01, 0.50*	0.02, 0.50*	0.03, 0.53*	0.03, 0.48*	0.03, 0.49*	0.06, 0.85*	0.06, 0.85*	0.06, 0.85*	0.04, 0.56*
<i>Pseudotsuga menziesii</i>	1.00 (0.14)	0.00, 1.00	0.00, 0.36*	0.00, 0.56*	0.11, 0.68*	0.09, 0.66*	0.00, 0.40*	0.00, 0.93*	0.00, 1.00	0.00, 1.00	0.00, 1.00	0.01, 0.82*
<i>Larix laricina</i>	0.50 (0.25)	0.00, 1.00	0.01, 0.40*	0.00, 0.67	0.04, 0.53	0.02, 0.51	0.00, 0.86	0.01, 0.62	0.00, 1.00	0.00, 1.00	0.00, 1.00	0.00, 0.84
<i>Abies concolor</i>	0.00 (0.11)	0.00, 1.00	0.01, 0.37*	0.03, 0.51	0.00, 0.44	0.00, 0.33	0.01, 0.49*	0.01, 0.39*	0.00, 1.00	0.00, 1.00	0.00, 1.00	0.00, 0.97
<i>Thuja occidentalis</i> ⁵	0.00 (0.12)	0.00, 1.00	0.00, 0.01	0.00, 1.00	0.42, 1.00*	0.76, 1.00*	0.00, 1.00	0.00, 1.00	0.00, 1.00	0.00, 1.00	0.00, 1.00	0.00, 1.00
<i>Sequoia sempervirens</i> group ⁶	0.00 (0.14)	0.00, 1.00	0.00, 0.01	0.00, 1.00	0.42, 1.00*	0.76, 1.00*	0.00, 1.00	0.00, 1.00	0.00, 1.00	0.00, 1.00	0.00, 1.00	0.00, 1.00

¹ data from Lee et al. (2008)

² includes *Pinus resinosa*, *P. canariensis*, *P. sylvestris*, *P. halepensis*, *P. sabiniana*, *P. strobus*, *P. pinea*, and *P. banksiana*

³ includes *Pinus jeffreyi*, *P. ponderosae*, and *P. lambertiana*

⁴ includes *Pinus radiata* and *P. taeda*

⁵ data from an independent experiment under quarantine conditions in St. Paul, MN

⁶ includes *Sequoia sempervirens* and *Calocedrus decurrens*

Table 5.10 Actual rate of boring into outer bark by *Orthotomicus erosus* when confined on tree species in the validation experiment and simultaneous 95% confidence intervals of the predicted rate of boring from the significant phylogenetic model. Confidence intervals that do not include the observed rate of population replacement are marked with an *.

Species	Rate of bark boring ¹	SE	95% Confidence Interval of the tamarack-only model
<i>Pinus resinosa</i>	0.51	0.03	0.35, 0.51
<i>Picea mariana</i>	0.63	0.03	0.34, 0.41*
<i>Pseudotsuga menziesii</i>	0.63	0.03	0.24, 0.38*
<i>Thuja occidentalis</i>	0.48	0.03	0.54, 1.00*

¹ data from an independent experiment under quarantine conditions in St. Paul, MN

Table 5.11 Genetic distances between species used in the experiments to generate the models of insect response and species used in the phylogenetic analysis for the *nad5* or *matk* genes.

Gene	Species from Experiments	GenBank Accession	Species in Phylogenetic analysis	Genetic Distance
<i>nad5</i>	<i>Abies balsamea</i>	AY159858	<i>Abies firma</i>	0.00368550
			<i>Picea abies</i>	0.00135135
	<i>Picea glauca</i>	DQ358177	<i>Picea smithiana</i>	0.00268456
			<i>Picea sitchensis</i>	0.00268456
			<i>Picea abies</i>	0.01085482
			<i>Picea smithiana</i>	0.01212938
	<i>Picea glauca</i>	AY196184	<i>Picea sitchensis</i>	0.01212938
			<i>Picea abies</i>	0.01085482
			<i>Picea smithiana</i>	0.01085482
	<i>Picea glauca</i>	AY196183	<i>Picea sitchensis</i>	0.01221167
<i>Picea abies</i>			0.01085482	
<i>Picea smithiana</i>			0.01085482	
<i>matk</i>	<i>Pinus resinosa</i>	AB080945	<i>Pinus armandii</i>	0.04608854

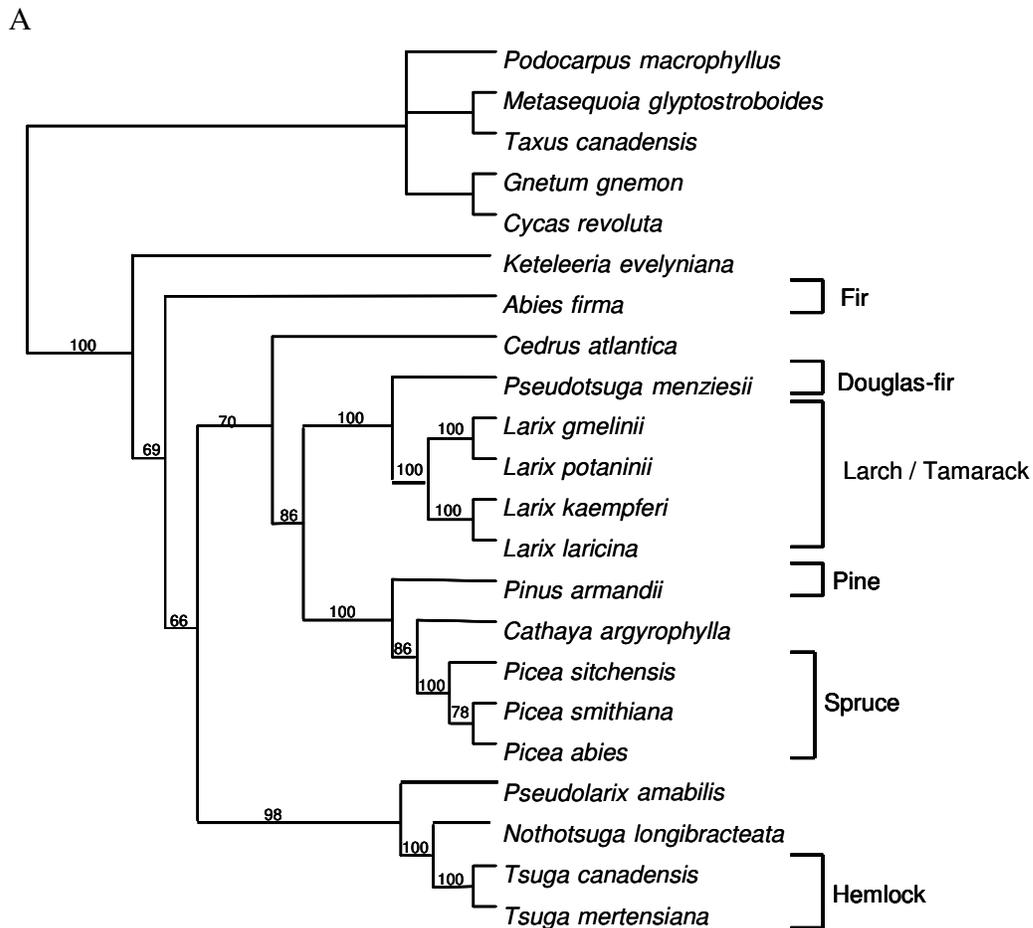
Table 5.12 Likelihood values of models of the probability of population replacement by a group of *O. erosus* forced into logs of various tree species generated by increasing or decreasing the phylogenetic distances to *Pinus* by 5% or taking the phylogenetic distance from *Larix laricina* instead of the base of *Larix*. *P* values are derived from a random permutation test. Significant models are marked with a *.

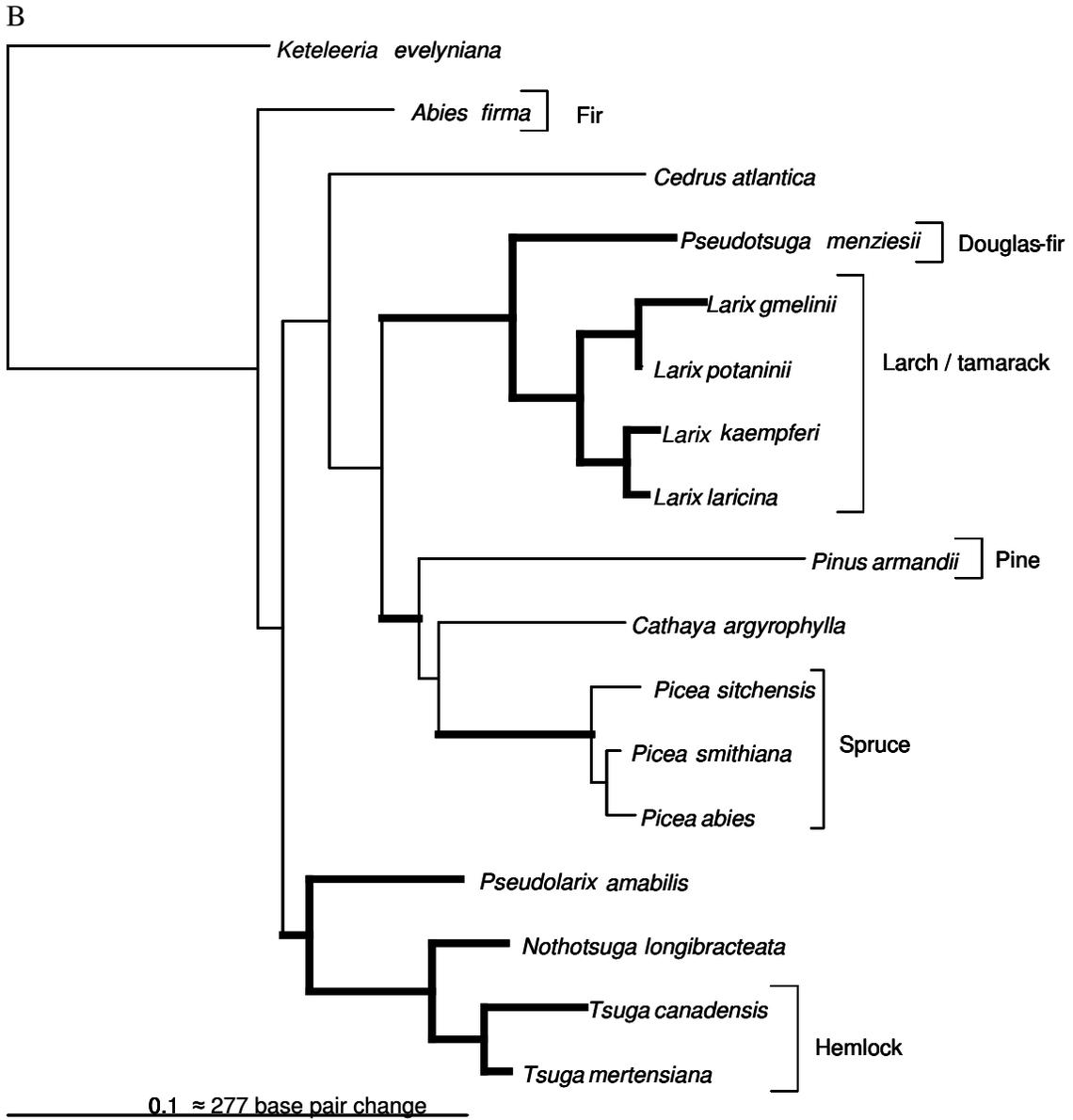
Model	Manipulation	Ln(likelihood)	<i>P</i>
Full	Increase <i>Pinus</i> 5%	-11.40	0.0001*
	Decrease <i>Pinus</i> 5%	-11.40	<0.0001*
	Distance from <i>Larix laricina</i>	-11.40	0.0001*
RP	Increase <i>Pinus</i> 5%	-14.34	<0.0001*
	Decrease <i>Pinus</i> 5%	-14.34	<0.0001*
T	Distance from <i>Larix laricina</i>	-17.16	0.0008*
RP + T	Increase <i>Pinus</i> 5%	-14.27	0.0007*
	Decrease <i>Pinus</i> 5%	-14.27	0.0003*
	Distance from <i>Larix laricina</i>	-14.27	0.0002*
RP + H	Increase <i>Pinus</i> 5%	-14.26	0.0003*
	Decrease <i>Pinus</i> 5%	-14.26	0.0002*
WS + T	Distance from <i>Larix laricina</i>	-11.40	<0.0001*
RP + WS	Increase <i>Pinus</i> 5%	-11.40	<0.0001*
	Decrease <i>Pinus</i> 5%	-11.40	<0.0001*
T + H + BF	Distance from <i>Larix laricina</i>	-13.22	0.0002*

Table 5.13 Likelihood values of models of the probability of boring into the outer bark by *O. erosus* confined on various tree species generated by increasing or decreasing the phylogenetic distance to *Pinus* by 5% or taking the phylogenetic distance from *Larix laricina* instead of the base of *Larix*. *P* values are derived from a random permutation test. Significant models are marked with a *.

Model	Manipulation	Ln(likelihood)	<i>P</i>
Full	Increase <i>Pinus</i> 5%	-388.17	0.0285
	Decrease <i>Pinus</i> 5%	-388.17	0.0260
	Distance from <i>Larix laricina</i>	-388.17	0.0271
RP	Increase 5%	-390.39	0.0106
	Decrease 5%	-390.39	0.0124
T	Distance from <i>Larix laricina</i>	-389.32	0.0047
RP + T	Increase 5%	-388.21	0.0051
	Decrease 5%	-388.21	0.0035*
	Distance from <i>Larix laricina</i>	-388.18	0.0039*
RP + H	Increase 5%	-389.52	0.0182
	Decrease 5%	-389.52	0.0169
WS + T	Distance from <i>Larix laricina</i>	-389.31	0.0121
RP + WS	Increase 5%	-390.36	0.0385
	Decrease 5%	-390.36	0.0352
T + H + BF	Distance from <i>Larix laricina</i>	-388.17	0.0131

Figure 5.1. A) Cladogram of Pinaceae and outgroup taxa displaying clade credibility values from a Bayesian analysis. B) Phylogram of the Pinaceae showing branch lengths between nodes. Branches with clade credibility >95 are in bold. Branch lengths to the outgroup were long, so the outgroup is excluded for ease of presentation.





Chapter 6: Steps Towards Predicting and Preventing Biological Invasions

Over the past century, invasive species in the United States have detrimentally affected all of the attributes of forest ecosystems...including biological diversity, forest health and productivity, water and soil quality, contribution to the carbon cycle, and socioeconomic values (Chornesky et al. 2005).

Prediction of the effects of specific invasive species and proactive management to prevent or slow their impact will provide many environmental and economic benefits. However, preventative management of an invasive species requires a large investment of resources and there are a very large number of potential invaders. Furthermore, only a small percentage of imported species will become damaging pests (Williamson 1996).

It is unlikely that resources will be available to develop and implement individual programs to prevent the introduction of every potentially invasive species, or that every potential invader could be anticipated. However, if species that will become successful invaders could be identified early in the invasion or before they establish, then management strategies for the most harmful species could be implemented (Worner 2002). Conversely, if ecosystems could be managed so as to be inherently less invasible to a number of species, this would also have the potential for preventing harm. The research presented in this dissertation does not fully accomplish either one of these goals, even for the single study insect. However, it does make some important steps towards both of them.

Prediction of the invasive potential of a species involves many considerations such as the propagule pressure, life history of the species (Kolar and Lodge 2001), climatic suitability (Worner 2002), and the presence of appropriate host plants (Baker 2002). While many of these factors will remain constant in native and introduced

populations (i.e., a freeze-intolerant insect will remain freeze intolerant), but host associations can be expected to change if the vegetation of the native and adventive ranges are different. Therefore, future host associations will need to be predicted. If the plant traits important in acceptability and suitability to insects are heritable, then acceptability and suitability should be predictable based on plant relatedness. This is a common assumption for methods such as the centripetal phylogenetic approach (Wapshere 1974) that have been successfully applied to specialist insects. However, oligophagous and polyphagous insects are more likely to be of concern as biological invaders. Host acceptability and suitability in these insect may be based on the absence of deterrents or toxins (Thorsteinson 1960, Jermy 1966) or the presence of phylogenetically disjunct characteristics (Becerra 1997, Agrawal and Fishbein 2006). In these cases, in depth understanding of the plant traits important for acceptability and suitability and knowledge of the distribution of those traits may be necessary (Payne et al. 2004).

An important result of the experiments presented here is that the set of species that are acceptable to *O. erosus* is broader than the set of suitable species. As a consequence, the beetles may accept unsuitable species even in the presence of suitable alternatives. These acceptable but unsuitable plants could then form a population sink, preventing establishment or affecting the future population dynamics of the population. This may explain why *O. erosus* has not been extensively reported from Monterey pine stands in California even though it is a pest of Monterey pine plantations in the rest of the world: other species present in the ecosystem may be forming a population sink. To

make an accurate prediction about the invasive potential in cases such as these, the relevant characteristics of both host and non-host species must be known.

Another important consequence of the mismatch between acceptability and suitability is that the presence of acceptable, unsuitable trees could make a habitat that has appropriate host plants less susceptible to invasion or decrease the impacts of an insect that is already present. This is the case for native populations of *Dendroctonus frontalis* Zimmerman, where Virginia pine is more often accepted than loblolly pine, even though loblolly pine is a more suitable host (Veysey et al. 2003). In this system, manipulating the proportions of these two species in managed forests has been suggested for insect control. Small populations of the gypsy moth are known to be more likely to persist in certain habitats (Tobin et al. 2007), and the effect of acceptable unsuitable plants is a possible mechanism for that effect. If many insect species will accept some novel host plants that are unsuitable, then managing areas where insects are likely to be introduced (i.e. ports or shipping and distribution centers) so that the surrounding vegetation is diverse could reduce the probability that a species would establish.

The presence of exotic plant species is another part of the ecosystem that could be managed to reduce the probability of invasion. Some of the main hosts used by *O. erosus* in its Californian range are pines introduced from the Mediterranean (Lee et al. 2005). It is possible that the beetle was able to establish because of the presence of the exotic pines and not because it immediately accepted suitable North American pines. Exotic parasitoids introduced for biological control are more likely to have been reported using a non-target native host if they had successfully established on the exotic. However, factors such as parasitoid life history, area of origin, habitat of introduction, life history of

the target, and time since introduction were not related to non-target host use (Hawkins and Marino 1997). The presence of host species from the native range therefore appears to influence the establishment potential of an exotic. Once established on the native hosts, exotic species may have a higher probability of moving on to native species. The maintenance of diverse native vegetation in areas where insects are likely to be introduced, such as urban forests (Liebhold et al. 1995), may reduce the probability that an exotic insect would be able to successfully establish in those areas, despite the presence of native plants that would be appropriate hosts.

Dealing with invasive insects on a case-by-case basis after they cause environmental and economic harm is becoming increasingly difficult as more and more species are introduced due to factors such as increasing global trade and climate change. The prevention of harm by these species is preferable. In order for successful prevention to take place, it will be necessary to manage ecosystems to minimize the potential establishment of any invader and develop accurate prediction and preventative management for those species with the highest damage potential. Although they will never replace field observations, studies of the potential invasive insects under quarantine conditions can help to develop these predictions and suggest both species-specific and ecosystem-wide management.

References

- Agosta, S. J. 2006.** On ecological fitting, plant-insect associations, herbivore host shifts, and host plant selection. *Oikos* 114: 556-565.
- Agrawal, A. A., and M. Fishbein. 2006.** Plant defense syndromes. *Ecology* 87: S132-S149.
- Akers, R. P., and D. L. Wood. 1989.** Olfactory orientation responses by walking female *Ips paraconfusus* bark beetles II. In an anemotaxis assay. *J Chem Ecol* 15: 1147-1159.
- Allan, F., D. Rollinson, J. E. Smith, and A. M. Dunn. 2009.** Host choice and penetration by *Schistosoma haematobium* miracidia. *J Helminthol* 83: 33-38.
- Amezaga, I., and M. A. Rodriguez. 1998.** Resource partitioning of four sympatric bark beetles depending on swarming dates and tree species. *Forest Ecol Manag* 109: 127-135.
- Arias, M., L. Robertson, A. Garcia-Alvarez, S. C. Arcos, M. Escuer, R. Sanz, and J. P. Mansilla. 2005.** *Bursaphelenchus fungivorus* (Nematoda: Aphelenchida) associate with *Orthotomicus erosus* (Coleoptera: Scolytidae) in Spain. *For. Path.* 35: 375-383.
- Badenes-Perez, F. R., B. A. Nault, and A. M. Shelton. 2006.** Dynamics of diamondback moth oviposition in the presence of a highly preferred non-suitable host. *Entomol Exp et Appl* 120: 23-31.
- Baker, R. H. A. 2002.** Predicting the limits to the potential distribution of alien crop pests, pp. 207-241. *In* G. J. Hallman and C. P. Schwalbe [eds.], *Invasive Arthropods in Agriculture: Problems and Solutions*. Science Publishers, Inc., Enfield, NH.
- Barre, F., F. Milsant, C. Palasse, V. Prigent, F. Goussard, and C. Geri. 2002.** Preference and performance of the sawfly *Diprion pini* on host and non-host plants of the genus *Pinus*. *Entomol Exp et Appl* 102: 229-237.

- Barton Browne, L., and T. M. Withers. 2002.** Time-dependent changes in the host-acceptance threshold of insects: Implications for host specificity testing of candidate biological control agents. *Biocontro Sci Techn* 12: 677-693.
- Becerra, J. X. 1997.** Insects on plants: macroevolutionary chemical trends in host use. *Science* 276: 253-256.
- Beck, S. D. 1965.** Resistance of plants to insects. *Annu. Rev. Entomol.* 10: 207-232.
- Bernays, E. A., and R. F. Chapman. 1994.** Host-plant selection by phytophagous insects. Chapman and Hall, New York.
- Bernays, E. A., and P. J. M. Minkenbergh. 1997.** Insect herbivores: different reasons for being a generalist. *Ecology* 78: 1157-1169.
- Borden, J. H., R. M. Silverstein, and R. G. Brownlee. 1968.** Sex pheromone of *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae): Production, bio-assay, and partial isolation. *Can Entomol* 100: 597-603.
- Brandl, M., and R. Brandl. 2006.** Is the composition of phytophagous insects and parasitic fungi among trees predictable? *Oikos* 113: 296-304.
- Brattli, J. G., J. Andersen, and A. C. Nilssen. 1998.** Primary attraction and host tree selection in deciduous and conifer living Coleoptera: Scolytidae, Curculionidae, Cerambycidae and Lymexylidae. *J. Appl. Ent.* 122: 345-352.
- Bright, D. E., and R. E. Skidmore. 1997.** A Catalog of Scolytidae and Platypodidae (Coleoptera), Supplement 1 (1990-1994). NRC Research Press, Ottawa, Ontario, Canada.
- Brockerhoff, E. G., A. M. Liebhold, and H. Jactel. 2006a.** The ecology of forest insect invasions and advances in their management. *Can J For Res* 36: 263-268.
- Brockerhoff, E. G., J. Bain, M. Kimberley, and M. Knizek. 2006b.** Interception frequency of exotic bark and ambrosia beetles (Coleoptera: Scolytinae) and relationship with establishment in New Zealand and worldwide. *Can. J. For. Res.* 36: 289-298.

- Brooks, D. R., V. Leon-Regagnon, D. A. McLennan, and D. Zelmer. 2006.** Ecological fitting as a determinant of the community structure of platyhelminth parasites of anurans. *Ecology* 87: S76-S85.
- Browne, L. E. 1972.** An emergence cage and refrigerated collector for wood-boring insects and their associates. *J. Econ. Entomol.* 65: 1499-1501.
- Browne, L. E., M. C. Birch, and D. L. Wood. 1974.** Novel trapping and delivery systems for airborne insect pheromones. *J Insect Physiol* 20: 183-193.
- Byers, J. A. 1989.** Behavioral mechanisms involved in reducing competition in bark beetles. *Holarctic Ecology* 12: 466-476.
- Byers, J. A., B. S. Lanne, J. Lofqvist, F. Schlyter, and G. Bergstrom. 1985.** Olfactory recognition of host-tree susceptibility by pine shoot beetles. *Naturwissenschaften* 72: 324-326.
- Campbell, S. A., and J. H. Borden. 2006.** Integration of visual and olfactory cues of hosts and non-hosts by three bark beetles (Coleoptera: Scolytidae). *Ecol. Entomol.* 31: 437-449.
- Casagrande, R. A., and J. E. Dacey. 2007.** Monarch butterfly oviposition on swallow-worts (*Vincetoxicum* spp.). *Environ Entomol* 36: 631-636.
- Cassell, D. L. 2002.** A randomization-test wrapper for SAS[®] PROCs, pp. 251. *In* C. Gareleck [ed.], SAS User's Group International Conference, April 14-17, 2002, Orlando, FL.
- Chararas, C., C. Revolon, M. Feinberg, and C. Ducauze. 1982.** Preference of certain Scolytidae for different conifers: a statistical approach. *J Chem Ecol* 8: 1093-1109.
- Chew, F. S. 1977.** Coevolution of Pierid butterflies and their cruciferous foodplants. II. The distribution of eggs on potential foodplants. *Evolution* 31: 568-579.
- Chornesky, E. A., A. M. Bartuska, G. H. Aplet, K. O. Britton, J. Cummings-Carlson, F. W. Davis, J. Eskow, D. R. Gordon, K. W. Gottschalk, R. A. Haack, A. J. Hansen, R. N. Mack, F. J. Rahel, M. A. Shannon, L. A.**

- Wainger, and T. B. Wigley. 2005.** Science priorities for reducing the threat of invasive species to sustainable forestry. *Bioscience* 55: 335-348.
- Copp, N. H., and D. Davenport. 1978.** *Agraulis* and *Passiflora* I. Control of specificity. *Biol Bull* 155: 98-112.
- Courtney, S., and T. Kibota. 1990.** Mother doesn't know best: Selection of hosts by ovipositing insects, pp. 161-188. *In* E. A. Bernays [ed.], *Insect-Plant Interactions*. CRC Press, Boca Raton.
- Courtney, S. P., G. K. Chen, and A. Gardner. 1989.** A general model for individual host selection. *Oikos* 55: 55-65.
- Dethier, V. G. 1982.** Mechanism of host-plant recognition. *Entomol Exp Appl* 31: 49-56.
- Dethier, V. G., B.-B. L, and C. N. Smith. 1960.** The designation of chemicals in terms of the responses they elicit from insects. *J Econ Entomol* 53: 134-136.
- Dixon, A. 1998.** *Aphid Ecology*. Chapman and Hall, London.
- Eglitis, A. 2000.** EXFOR Database pest reports: *Orthotomicus erosus*. USDA Forest Service. Available on-line at:
<http://spfnic.fs.fed.us/exfor/data/pestreports.cfm?pestidval=9&langdisplay=english>
h. Accessed 21 September 2007.
- Elkinton, J. S., and D. L. Wood. 1980.** Feeding and boring behavior of the bark beetle *Ips paraconfusus* (Coleoptera: Scolytidae) on the bark of a host and non-host tree species. *Can Entomol* 112: 797-809.
- Elkinton, J. S., D. L. Wood, and L. B. Hendry. 1980.** Pheromone production by the bark beetle, *Ips paraconfusus*, in the nonhost, white fir. *J. Chem. Ecol.* 6: 979-987.
- Elkinton, J. S., D. L. Wood, and L. E. Browne. 1981.** Feeding and boring behavior of the bark beetle, *Ips paraconfusus*, in extracts of ponderosa pine phloem. *J Chem Ecol* 7: 209-220.

- Erbilgin, N., S. R. Mori, J. H. Sun, J. D. Stein, D. R. Owen, L. D. Merrill, R. C. Bolanos, K. F. Raffa, T. M. Montiel, D. L. Wood, and N. E. Gillette. 2007.** Response to host volatiles by native and introduced populations of *Dendroctonus valens* (Coleoptera: Curculionidae, Scolytinae) in North America and China. *J Chem Ecol* 33: 131-146.
- Ernst, C., N. Cappuccino, and J. T. Arnason. 2007.** Potential novel hosts for the lily leaf beetle *Lilioceris lili* Scopoli (Coleoptera: Chrysomelidae) in eastern North America. *Ecological Entomology* 32: 45-52.
- Faccoli, M., G. Anfora, and M. Tasin. 2008.** Responses of the Mediterranean pine shoot beetle *Tomicus destruens* (Wollaston) to pine shoot and bark volatiles. *J Chem Ecol* 34: 1162-1169.
- Felsenstein, J. 1985.** Phylogenies and the comparative method. *Am Nat* 125: 1-15.
- Furniss, M. M. 1976.** Controlled breeding, comparative anatomy and bionomics of *Dendroctonus simplex* LeConte and *Dendroctonus pseudotsugae* Hopkins (Coleoptera: Scolytidae), pp. 109-120. *In* W. G. Barr [ed.], Department of Entomology Anniversary Publication 15. University of Idaho.
- Gall, R., W. Landolt, P. Schleppei, V. Michellod, and J. B. Bucher. 2002.** Water content and bark thickness of Norway spruce (*Picea abies*) stems: phloem water capacitance and xylem sap flow. *Tree Physiol* 22: 613-623.
- Gast, S. J., M. W. Stock, and M. M. Furniss. 1993.** Physiological factors affecting attraction of *Ips pini* (Coleoptera: Scolytidae) to host odor or natural male pheromone in Idaho. *Ann Entomol Soc Am* 86: 417-422.
- Giesen, V. H., U. Kohnle, J. P. Vite, M. L. Pan, and W. Francke. 1984.** Das aggregationspheromon des mediterranen Kiefernborke-käfers *Ips* (*Orthotomicus*) *erosus*. *Z. ang. Ent.* 98: 95-97.
- Gobner, M. M., A. Chao, R. I. Bailey, and A. Prinzing. 2009.** Native fauna on exotic trees: Phylogenetic conservatism and geographic contingency in two lineages of phlophages on two lineages of trees. *Am Nat* 173: 599-614.

- Gratton, C., and S. C. Welter. 1998.** Oviposition preference and larval performance of *Liriomyza helianthi* (Diptera: Agromyzidae) on normal and novel host plants. *Environ Entomol* 27: 926-935.
- Graves, A. D., E. H. Holsten, M. E. Ascerno, K. P. Zogas, J. S. Hard, D. P. W. Huber, R. A. Blanchette, and S. J. Seybold. 2008.** Protection of spruce from colonization by the bark beetle, *Ips perturbatus*, in Alaska. *For Ecol Manag* 256: 1825-1839.
- Graves, S. D., and A. M. Shapiro. 2003.** Exotics as host plants of the California butterfly fauna. *Biol Conserv* 110: 413-433.
- Gripenberg, S., E. Morrien, A. Cudmore, J.-P. Salminen, and T. Roslin. 2007.** Resource selection by female moths in a heterogenous environment: what is a poor girl to do? *J Anim Ecol* 76: 854-865.
- Guerrero, A., J. Feixas, J. Pajares, L. J. Wadhams, J. A. Pickett, and C. M. Woodcock. 1997.** Semiochemically induced inhibition of behaviour of *Tomicus destruens* (Woll.) (Coleoptera: Scolytidae). *Naturwissenschaften* 84: 155-157.
- Haack, R. A. 2006.** Exotic bark- and wood-boring Coleoptera in the United States: recent establishments and interceptions. *Can J For Res* 36: 269-288.
- Harris, M., M. Sandanayaka, and W. Griffin. 2001.** Oviposition preferences of the Hessian fly and their consequences for the survival and reproductive potential of offspring. *Ecol Entomol* 26: 473-486.
- Hawkins, B. A., and P. C. Marino. 1997.** The colonization of native phytophagous insects in North America by exotic parasitoids. *Oecologia* 112: 566-571.
- Hovorka, O., J. Kindl, B. Kalinova, M. Knizek, P. Vrkocova, and B. Koutek. 2005.** The role of beetle and host volatiles in host colonization in the European oak bark beetle, *Scolytus intricatus* (Ratzeburg) (Col., Scolytidae). *J. Appl. Entomol.* 129: 221-226.
- Huber, D. P. W., R. Gries, J. H. Borden, and H. D. J. Pierce. 2000.** A survey of antennal responses by five species of coniferophagous bark beetles (Coleoptera: Scolytidae) to bark volatiles of six species of angiosperm trees. *Chemoecology* 10: 103-113.

- Huelsenbeck, J. P., and F. Ronquist. 2001.** MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.
- Hynum, B. G., and A. A. Berryman. 1980.** *Dendroctonus ponderosae* (Coleoptera: Scolytidae): Pre-aggregation landing and gallery initiation on lodgepole pine. *Can Entomol* 112: 185-191.
- Ishihara, M., and T. Ohgushi. 2008.** Enemy-free space? Host preference and larval performance of a willow leaf beetle. *Popul Ecol* 50: 35-43.
- Jaenike, J. 1990.** Host specialization in phytophagous insects. *Annu Rev Ecol Syst* 21: 243-273.
- Janz, N. 2002.** Evolutionary ecology of oviposition strategies, pp. 349-376. *In* M. Hilker and T. Meiners [eds.], *Chemoecology of Insect Eggs and Egg Deposition*. Blackwell Publishing Company, Berlin.
- Janz, N., K. Nyblom, and S. Nylin. 2001.** Evolutionary dynamics of host-plant specialization: A case study of the tribe Nymphalini. *Evolution* 55: 783-796.
- Janzen, D. H. 1985.** On ecological fitting. *Oikos* 45: 308-310.
- Jermey, T. 1966.** Feeding inhibitors and food preference in chewing phytophagous insects. *Entomol Exp Appl* 9: 1-12.
- Jermey, T. 1984.** Evolution of insect/host plant relationships. *American Naturalist* 124: 609-630.
- Jermey, T. 1988.** Can predation lead to narrow food specialization in phytophagous insects? *Ecology* 69: 902-904.
- Kearney, M., and J. M. Clark. 2003.** Problems due to missing data in phylogenetic analyses including fossils: A critical review. *J Vertebr Paleontol* 23: 263-274.
- Kennedy, J. S. 1965.** Mechanisms of host plant selection. *Ann Appl Biol* 56: 317-322.

- Kergoat, G. J., A. Delobel, G. Fediere, B. Le Ru, and J. F. Silvain. 2005.** Both host-plant phylogeny and chemistry have shaped the African seed-beetle radiation. *Mol Phylogenet Evol* 35: 602-611.
- Kergoat, G. J., J. F. Silvain, A. Delobel, M. Tuda, and K.-W. Anton. 2007.** Defining the limits of taxonomic conservatism in host-plant use for phytophagous insects: Molecular systematics and evolution of host-plant associations in the seed-beetle genus *Bruchus* Linnaeus (Coleoptera: Chrysomelidae: Bruchinae). *Mol Phylogenet Evol* 43: 251-269.
- Klepzig, K. D., E. B. Smalley, and K. F. Raffa. 1996.** Combined chemical defenses against an insect-fungal complex. *J Chem Ecol* 22: 1367-1388.
- Kolar, C. S., and D. M. Lodge. 2001.** Progress in invasion biology: predicting invaders. *Trends Ecol Evol* 16: 199-204.
- Kuehl, R. O. 2000.** *Design of Experiments: Statistical Principles of Research Design and Analysis.* Thompson Learning, Pacific Grove, CA.
- Kvanli, A. H. 1988.** *Statistics: A computer integrated approach.* West Publishing Co, St Paul, MN.
- Larsson, S., and B. Ekbom. 1995.** Oviposition mistakes in herbivorous insects: confusion or a step towards a new host plant? *Oikos* 72: 155-160.
- Leahy, M. J. A., T. H. Oliver, and S. R. Leather. 2007.** Feeding behaviour of the black pine beetle, *Hylastes ater* (Coleoptera: Scolytidae). *Agric For Entomol* 9: 115-124.
- Lee, J. C., S. L. Smith, and S. J. Seybold. 2005.** Mediterranean Pine Engraver. USDA Forest Service Pest Alert R5-PR-016: 4 pp.
- Lee, J. C., M. L. Flint, and S. J. Seybold. 2008.** Suitability of pines and other conifers as hosts for the invasive Mediterranean pine engraver (Coleoptera: Scolytidae) in North America. *J Econ Entomol* 101: 829-837.

- Lee, J. C., R. A. Haack, J. F. Negrón, J. J. Witcosky, and S. J. Seybold. 2007.** Invasive bark beetles. USDA Forest Service Forest Insect Disease Leaflet 176: 12 pp.
- Lee, R. E. 1991.** Principles of insect low temperature tolerance, pp. 17-46. *In* R. E. Lee Jr. and D. L. Denlinger [eds.], *Insects at Low Temperature*. Chapman and Hall, New York.
- Liebert, A. E., and P. T. Starks. 2004.** The action component of recognition systems: a focus on the response. *Ann Zool Fennici* 41: 747-764.
- Liebhold, A. M., W. L. MacDonald, D. Bergdahl, and V. C. Mastro. 1995.** Invasion by exotic forest pests: A threat to forest ecosystems. *Forest Science Monograph* 30: 1-49.
- Macias-Samano, J. E., J. H. Borden, R. Gries, H. D. Pierce Jr., G. Gries, and G. G. S. King. 1998.** Primary attraction of the fir engraver, *Scolytus ventralis*. *J Chem Ecol* 24: 1049-1075.
- Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000.** Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecol Appl* 10: 689-710.
- Manly, B. F. J. 2007.** *Randomization, Bootstrap and Monte Carlo Methods in Biology*. Chapman & Hall / CRC, Boca Raton, FL.
- Mayhew, P. J. 1997.** Adaptive patterns of host-plant selection by phytophagous insects. *Oikos* 79: 417-428.
- McNee, W. R., P. Bonello, A. J. Storer, D. L. Wood, and T. R. Gordon. 2003.** Feeding response of *Ips paraconfusus* to phloem and phloem metabolites of *Heterobasidion annosum*-innoculated ponderosa pine *Pinus ponderosa*. *J Chem Ecol* 29: 1183-1202.
- McPheron, L. J., S. J. Seybold, A. J. Storer, D. L. Wood, T. Ohtsuka, and I. Kubo. 1997.** Effects of enantiomeric blend of verbenone on response of *Ips paraconfusus* to naturally produced aggregation pheromone in the laboratory. *J. Chem. Ecol.* 23: 2825-2839.

- Mendel, Z. 1983.** Seasonal history of *Orthotomicus erosus* (Coleoptera: Scolytidae) in Israel. *Phytoparasitica* 11: 13-24.
- Mendel, Z. 1988.** Attraction of *Orthotomicus erosus* and *Pityogenes calcaratus* to a synthetic aggregation pheromone of *Ips typographus*. *Phytoparasitica* 16: 109-117.
- Mendel, Z., and J. Halperin. 1982.** The biology and behavior of *Orthotomicus erosus* in Israel. *Phytoparasitica* 10: 169-181.
- Mendel, Z., Z. Madar, and Y. Golan. 1985.** Comparison of the seasonal occurrence and behavior of seven pine bark beetles (Coleoptera: Scolytidae) in Israel. *Phytoparasitica* 13: 21-32.
- Mendel, Z., O. Boneh, Y. Shenhar, and J. Riov. 1991.** Diurnal flight patterns of *Orthotomicus erosus* and *Pityogenes calcaratus* in Israel. *Phytoparasitica* 19: 23-31.
- Miller, J. R., and K. L. Strickler. 1984.** Finding and accepting host plants, pp. 127-157. *In* W. J. Bell and R. T. Carde [eds.], *Chemical Ecology of Insects*. Chapman and Hall Ltd., London.
- Miller, J. S. 1987.** Host-plant relationship in the Papilionidae (Lepidoptera): Parallel cladogenesis or colonization? *Cladistics* 3: 105-120.
- Mitter, C., and B. Farrell. 1991.** Macroevolutionary aspects of insect-plant relationships, pp. 35-78. *In* E. A. Bernays [ed.], *Insect-Plant Interactions*. CRC Press, Boca Raton.
- Moeck, H. A. 1970.** An olfactometer for the bio-assay of attractants for scolytids. *Can. Entomol.* 102: 792-796.
- Moeck, H. A., D. L. Wood, and K. Q. Lindahl Jr. 1981.** Host selection behavior of bark beetles (Coleoptera: Scolytidae) attacking *Pinus ponderosa*, with special emphasis on the western pine beetle, *Dendroctonus brevicomis*. *J. Chem. Ecol.* 7: 49-83.

- Nylander, J. A. A. 2004.** MrModeltest 2.3 computer program, version 2.3. By Nylander, J. A. A., Evolutionary Biology Centre, Uppsala University.
- O'Neal, M. E., D. A. Landis, and R. Isaacs. 2002.** An inexpensive, accurate method for measuring leaf area and defoliation through digital image analysis. *J Econ Entomol* 95: 1190-1194.
- Odegaard, F., O. H. Diserud, and K. Ostbye. 2005.** The importance of plant relatedness for host utilization among phytophagous insects. *Ecol Lett* 8: 612-617.
- Ojala, K., R. Julkunen-Titto, L. Lindstrom, and J. Mappes. 2005.** Diet affects the immune defence and life-history traits of an Arctiid moth *Parasemia pantaginis*. *Evolutionary Ecology Research* 7: 1153-1170.
- Ott, R. L., and M. Longnecker. 2001.** An Introduction to Statistical Methods and Data Analysis. Duxbury, Pacific Grove, CA.
- Page, R. D. M. 1996.** TREEVIEW: An application to display phylogenetic trees on personal computers. *Comput Appl Biosci* 12: 357-358.
- Payne, C. M., C. V. Tillberg, and A. V. Suarez. 2004.** Recognition systems and biological invasions. *Ann Zool Fennici* 41: 843-858.
- Pemberton, R. W. 2000.** Predictable risk to native plants in weed biological control. *Oecologia* 125: 489-494.
- Person, H. L. 1931.** Theory in explanation of the selection of certain trees by the western pine beetle. *J. For.* 29: 696-699.
- Pinski, R. A., W. J. Mattson, and K. F. Raffa. 2005.** Host breadth and ovipositional behaviour of adult *Polydrusus sericeus* and *Phyllobius oblongus* (Coleoptera: Curculionidae), nonindigenous inhabitants of northern hardwood forests. *Environ Entomol* 34: 148-157.
- Pokon, R., V. Novotny, and G. A. Samuelson. 2005.** Host specialization and species richness of root-feeding chrysomelid larvae (Chrysomelidae, Coleoptera) in a New Guinea rain forest. *J Tropical Ecol* 21: 595-604.

- Pureswaran, D. S., and J. H. Borden. 2003.** Test of semiochemical mediated host specificity in four species of tree killing bark beetles (Coleoptera: Scolytidae). *Environ Entomol* 32: 963-969.
- Pureswaran, D. S., and J. H. Borden. 2005.** Primary attraction and kairomonal host discrimination in three species of *Dendroctonus* (Coleoptera: Scolytidae). *Agric. For. Entomol.* 7: 219-230.
- Pureswaran, D. S., R. Gries, and J. H. Borden. 2004.** Quantitative variation in monoterpenes in four species of conifers. *Biochem Syst Ecol* 32: 1109-1136.
- Raffa, K. F. 1988.** Host orientation behavior of *Dendroctonus ponderosae*: Integration of token stimuli host and defenses, pp. 369-390. *In* W. J. Mattson, J. Levieux and C. Bernard-Dagan [eds.], *Mechanisms of Woody Plant Defenses Against Insects: Search for Pattern*. Springer-Verlag, New York.
- Raffa, K. F., and A. A. Berryman. 1982.** Gustatory cues in the orientation of *Dendroctonus ponderosae* (Coleoptera: Scolytidae) to host trees. *Can Entomol* 114: 97-104.
- Renwick, J. A. A. 2001.** Variable diets and changing taste in plant-insect relationships. *J Chem Ecol* 27: 1063-1076.
- Riffell, J., L. Abrell, and J. Hildebrand. 2008.** Physical processes and real-time chemical measurement of the insect olfactory environment. *J. Chem. Ecol.* 34: 837-843.
- Rodman, J. E., and F. S. Chew. 1980.** Phytochemical correlates of herbivory in a community of native and naturalized Cruciferae. *Biochem Syst Ecol* 8: 43-50.
- Roininen, H., and J. Tahvanainen. 1989.** Host selection and larval performance of two willow-feeding sawflies. *Ecology* 70: 129-136.
- Ronquist, F., and J. P. Huelsenbeck. 2003.** MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

- Roques, A., M.-A. Auger-Rozenberg, and S. Boivin. 2006.** A lack of native congeners may limit colonization of introduced conifers by indigenous insects in Europe. *Can J For Res* 36: 299-313.
- Salom, S. M., J. A. Carlson, B. N. Ang, D. M. Grosman, and E. R. Day. 1994.** Laboratory evaluation of biologically-based compounds as antifeedants for the Pales weevil, *Hylobius pales* (Herbst) (Coleoptera: Curculionidae). *J Entomol Sci* 29: 407-419.
- SAS Institute Inc. 2004.** SAS Version 9.1. SAS Institute, Cary, NC.
- Sauvard, D. 2004.** General biology of bark beetles, pp. 63-88. *In* F. Lieutier, K. R. Day, A. Battisti, J. C. Gregoire and H. F. Evans [eds.], *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*. Kluwer Academic Publishers, Dordrecht.
- Schaffner, U. 2001.** Host range testing of insects for biological weed control: how can it be better interpreted? *Bioscience* 51: 951-959.
- Scheirs, J., and L. De Bruyn. 2002.** Integrating optimal foraging and optimal oviposition theory in plant-insect research. *Oikos* 96: 187-191.
- Schlaepfer, M. A., P. W. Sherman, B. Blossey, and M. C. Runge. 2005.** Introduced species as evolutionary traps. *Ecology Letters* 8: 241-246.
- Schlyter, F., E. Marling, and J. Löfqvist. 2004.** A new microassay for antifeedants in *Hylobius* pine weevils (Coleoptera). *J Pest Sci* 77: 191-195.
- Schoonhoven, L. M., T. Jermy, and J. J. A. van Loon. 1998.** *Insect-Plant Biology From Physiology to Evolution*. Chapman and Hall, New York.
- Schoonhoven, L. M., J. J. A. van Loon, and M. Dicke. 2005.** *Insect-Plant Biology*. Oxford University Press, New York.
- Schroeder, L. M. 1992.** Olfactory recognition of nonhosts aspen and birch by conifer bark beetles *Tomicus piniperda* and *Hylurgops palliatus*. *J. Chem. Ecol.* 18: 1583-1593.

- Seybold, S. J., and M. Downing. 2009.** What risk do invasive bark beetles and woodborers pose to forests of the western U.S.?: A case study of the Mediterranean pine engraver, *Orthotomicus erosus*. In J. L. Hayes and J. E. Lundquist [eds.], The Western Bark Beetle Research Group: A Unique Collaboration with Forest Health Protection: A Workshop at the 2007 Society of American Foresters National Convention. USDA Forest Service Gen Tech Rep GTR-PNW-784, Portland, OR.
- Seybold, S. J., and M. Downing, in press.** What risk do invasive bark beetles and woodborers pose to forests of the western U.S.?: A case study of the Mediterranean pine engraver, *Orthotomicus erosus*. In J. L. Hayes and J. E. Lundquist [eds.], The Western Bark Beetle Research Group: A Unique Collaboration with Forest Health Protection: A Workshop at the 2007 Society of American Foresters National Convention. USDA Forest Service Gen Tech Rep GTR-PNW-784, Portland, OR.
- Seybold, S. J., D. P. W. Huber, J. C. Lee, A. D. Graves, and J. Bohlmann. 2006.** Pine monoterpenes and pine bark beetles: a marriage of convenience for defense and chemical communication. *Phytochem. Rev.* 5: 143-178.
- Shepherd, W. P., D. P. W. Huber, S. J. Seybold, and C. J. Fettig. 2007.** Antennal responses of the western pine beetle, *Dendroctonus brevicomis* (Coleoptera: Curculionidae), to stem volatiles of its primary host, *Pinus ponderosa*, and nine sympatric nonhost angiosperms and conifers. *Chemoecology* 17: 209-221.
- Stastny, M., A. Battisti, E. Petrucco-Toffolo, F. Schlyter, and S. Larsson. 2006.** Host-plant use in the range expansion of the pine processionary moth, *Thaumetopoea pityocampa*. *Ecol. Entomol.* 31: 481-490.
- Straatman, R. 1962.** Notes on certain Lepidoptera ovipositing on plants which are toxic to their larvae. *J Lepid Soc* 16: 99-103.
- Swofford, D. L. 2003.** PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland, MA.
- Symons, F. B., and G. W. Beccaloni. 1999.** Phylogenetic indices for measuring the diet breadths of phytophagous insects. *Oecologia* 119: 427-434.
- Tallamy, D. W. 2000.** Physiological issues in host range expansion, pp. 11-26. In R. Van Driesche [ed.], X International Symposium on Biological Control of Weeds,

Bozeman, Montana, USA, July 4-14, 1999: Proceedings: Host Specificity Testing of Exotic Arthropod Biological Control Agents: The Biological Basis for Improvement in Safety. USDA Forest Service, Forest Health Technology Enterprise Team.

Teale, S. A., and G. N. Lanier. 1991. Seasonal variability in response of *Ips pini* (Coleoptera: Scolytidae) to ipsdienol in New York. *J. Chem. Ecol.* 17: 1145-1158.

Teale, S. A., F. X. Webster, A. Zhang, and G. N. Lanier. 1991. Lanierone: a new pheromone component from *Ips pini* (Coleoptera: Scolytidae) in New York. *J. Chem. Ecol.* 17: 1159-1176.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673-4680.

Thompson, J. N., W. Wehling, and R. Podolsky. 1990. Evolutionary genetics of host use in swallowtail butterflies. *Nature* 344: 148-150.

Thorsteinson, A. J. 1960. Host selection in phytophagous insects. *Annu Rev Entomol* 5: 193-218.

Tobin, P. C., S. L. Whitmire, D. M. Johnson, O. N. Bjornstad, and A. M. Liebhold. 2007. Invasion speed is affected by geographical variation in the strength of Allee effects. *Ecol Lett* 10: 36-43.

Tunset, K., A. C. Nilssen, and J. Andersen. 1993. Primary attraction in host recognition of coniferous bark beetles and bark weevils (Col., Scolytidae and Curculionidae). *J. Appl. Ent.* 115: 155-169.

Venette, R. C., A. J. Walter, and S. J. Seybold. 2008. Comparing risks from native and exotic bark beetles to the health of Great Lakes forests, Society of American Foresters 2008 Annual Meeting Proceedings. On CD. Available online at: <http://www.x-cdtech.com/SAF2008/prof230.html>.

Veysey, J. S., Matthew P. Ayres, Maria J. Lombardero, Richard W Hofstetter, and K. Kier D. 2003. Relative suitability of Virginia pine and loblolly pine as host

species for *Dendroctonus frontalis* (Coleoptera: Scolytidae). Environ. Entomol. 32: 668-679.

Vinson, S. B., and G. F. Iwantsch. 1980. Host suitability for insect parasitoids. Ann Rev Entomol 25: 397-419.

Vitousek, P. M., C. M. D'Antonio, L. L. Loope, M. Rejmanek, and R. Westbrooks. 1997. Introduced species: A significant component of human-caused global change. New Zeal J Ecol 21: 1-16.

Wallin, K. F., and K. F. Raffa. 2000. Influences of host chemicals and internal physiology on the multiple steps of postlanding host acceptance behaviour of *Ips pini* (Coleoptera: Scolytidae). Environ Entomol 29: 442-453.

Wallin, K. F., and K. F. Raffa. 2002. Prior encounters modulate subsequent choices in host acceptance behaviour by the bark beetle *Ips pini*. Entomol Exp App 103: 205-218.

Wallin, K. F., and K. F. Raffa. 2004. Feedback between individual host selection behavior and population dynamics in an eruptive herbivore. Ecol Monogr 74: 101-116.

Wallin, K. F., J. Rutledge, and K. F. Raffa. 2002. Heritability of host acceptance and gallery construction behaviors of the bark beetle *Ips pini* (Coleoptera: Scolytidae). Environ Entomol 31: 1276-1281.

Walter, A. J., E. M. Albrecht, and R. C. Venette. 2008. *Orthotomicus erosus*, pp. 90-100. In R. C. Venette [ed.], Pine Commodity-based Survey Reference, Cooperative Agriculture Pest Survey, U.S. Department of Agriculture, Animal Plant Health Inspection Service.

Walter, A. J., R. C. Venette, and S. A. Kells. in review-a. Acceptance and suitability of novel North American hosts for *Orthotomicus erosus*, a potential invasive bark beetle. Biol Inv

Walter, A. J., R. C. Venette, S. A. Kells, and S. J. Seybold. in review-b. Walking response of the Mediterranean pine engraver, *Orthotomicus erosus*, to host and non-host volatiles in a laboratory olfactometer. J Insect Behav.

- Wang, X.-Q., D. C. Tank, and T. Sang. 2000.** Phylogeny and divergence times in Pinaceae: evidence from three genomes. *Mol Biol Evol* 17: 773-781.
- Wapshere, A. J. 1974.** A strategy for evaluating the safety of organisms for biological weed control. *Ann Appl Biol* 77: 201-211.
- Warren, C. E., D. L. Wood, S. J. Seybold, A. J. Storer, and W. E. Bros. 1996.** Olfactory responses of *Ips plastographus maritimus* Lanier (Coleoptera: Scolytidae) to insect and host-associated volatiles in the laboratory. *J. Chem. Ecol.* 22: 2299-2316.
- Weisberg, S. 2005.** Applied Linear Regression. Wiley Interscience, Hoboken, New Jersey.
- Wiens, J. J. 2003.** Missing data, incomplete taxa, and phylogenetic accuracy. *Syst Biol* 52: 528-538.
- Wiklund, C. 1975.** The evolutionary relationship between adult oviposition preferences and larval host plant range in *Papilio machaon* L. *Oecologia* 18: 185-197.
- Williamson, M. 1996.** Biological Invasions. Chapman & Hall, London.
- Wood, D. L. 1962.** The attraction created by males of a bark beetle *Ips confusus* (LeConte) attacking ponderosa pine. *Pan-Pacific Entomol.* 38: 141-145.
- Wood, D. L. 1963.** Studies on host selection by *Ips confusus* (LeConte) (Coleoptera: Scolytidae) with special reference to Hopkins' host selection principle. University of California Publications in Entomology 27: 241-282.
- Wood, D. L. 1970.** Pheromones of bark beetles, pp. 301-316. In D. L. Wood, R. M. Silverstein and M. Nakajima [eds.], Control of Insect Behavior by Natural Products. Academic Press, New York, NY.
- Wood, D. L. 1982a.** The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. *Annu. Rev. Entomol.* 27: 411-446.

- Wood, D. L., and R. W. Bushing. 1963.** The olfactory response of *Ips confusus* (LeConte) (Coleoptera: Scolytidae) to the secondary attraction in the laboratory. *Can. Entomol.* 95: 1066-1078.
- Wood, S. L. 1982b.** The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. *Great Basin Naturalist Memoirs* 6: 1-1359.
- Wood, S. L. 2007.** Bark and Ambrosia Beetles of South America (Coleoptera, Scolytidae). Brigham Young University, M.L. Bean Life Science Museum, Provo, Utah.
- Wood, S. L., and D. E. Bright. 1992.** A catalog of Scolytidae and Platypodidae, Part 2, Taxonomic Index, Volume A and B. *Great Basin Nat Mem* 13: 1-833.
- Worner, S. P. 2002.** Predicting the invasive potential of exotic insects, pp. 119-137. *In* G. J. Hallman and C. P. Schwalbe [eds.], *Invasive Arthropods in Agriculture Problems and Solutions*. Science Publishers, Inc., Enfield, NH.
- Wyckhuys, K. A. G., and G. E. Heimpel. 2007.** Response of the soybean aphid parasitoid *Binodoxys communis* to olfactory cues from target and non-target host-plant complexes. *Entomol. Exp. App.* 123: 149-158.
- Zhang, L.-W., N. E. Gillette, and J.-H. Sun. 2007.** Electrophysiological and behavioral responses of *Dendroctonus valens* to non-host volatiles. *Ann. For. Sci.* 64: 267-273.
- Zhang, Q. H., and F. Schlyter. 2004.** Olfactory recognition and behavioral avoidance of angiosperm nonhost volatiles by conifer-inhabiting bark beetles. *Agric For Entomol* 6: 1-19.
- Zhang, Q. H., J. A. Byers, and F. Schlyter. 1992.** Optimal attack density in the larch bark beetle, *Ips cembrae* (Coleoptera: Scolytidae). *J Anim Ecol* 29: 672-678.
- Zhang, Q. H., F. Schlyter, and G. Birgersson. 2000.** Bark volatiles from nonhost angiosperm trees of spruce bark beetle, *Ips typographus* (L.) (Coleoptera: Scolytidae): Chemical and electrophysiological analysis. *Chemoecology* 10: 69-80.

Zhang, Q. H., T. Tolasch, F. Schlyter, and W. Francke. 2002. Enantiospecific antennal response of bark beetles to spiroacetal (*E*)-conophthorin. *J. Chem. Ecol.* 28: 1839-1852.

Zwolfer, H., and P. Harris. 1971. Host specificity determination of insects for biological control of weeds. *Annu. Rev. Entomol.* 16: 159-178.