

Temptations of weevil: feeding and ovipositional behaviour of *Hylobius warreni* Wood on host and nonhost bark in laboratory bioassays

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- Abstract**
- 1 Warren root collar weevil *Hylobius warreni* Wood (Coleoptera: Curculionidae) is a long-lived, flightless insect native to coniferous forests across northern North America. Girdling by larval feeding causes significant mortality on young trees. The insect poses considerable challenges to reforestation.
 - 2 Adult weevils feed on all life stages of a variety of coniferous hosts prior to oviposition. Their relative feeding preferences, however, have not been quantified. Moreover, it is not known whether host bark influences oviposition behaviour.
 - 3 Feeding preferences of adult weevils were tested in both choice and no-choice laboratory bioassays using small branches from three conifers (lodgepole pine *Pinus contorta* var. *latifolia*, interior hybrid spruce *Picea glauca* × *engelmannii*, and Douglas-fir *Pseudotsuga menziesii*) and one deciduous tree (trembling aspen *Populus tremuloides*). Measurements included the surface area of bark consumed, rate of consumption, the number of days of feeding, and, in the no-choice assay, the number of eggs oviposited.
 - 4 Bark consumption was greatest on pine and Douglas-fir, followed by spruce. Little to no feeding occurred on aspen. Consumption did not vary between male versus female insects for any of the feeding metrics quantified.
 - 5 The presence of aspen branches did not inhibit feeding on any of the other species in the choice bioassays.
 - 6 The number of eggs laid by female insects did not differ significantly among tree species in the no-choice assay. Eggs were laid indiscriminately in the presence of all four host types.
 - 7 Results and opportunities for future research are discussed in the context of formulating new integrated pest management strategies for this insect, which is increasingly important in the period of reforestation subsequent to the mountain pine beetle epidemic in western Canada.

Keywords Choice assay, Coleoptera, Curculionidae, Douglas-fir, feeding behaviour, feeding preferences, host selection, interior hybrid spruce, lodgepole pine, no-choice assay, trembling aspen, Warren root collar weevil.

Introduction

Below-ground herbivores play important roles in forest ecosystems, influencing gap formation, succession and nutrient cycling. In forest production systems, economic losses to root

feeders may be quite severe and may pose special challenges to forest management. Root herbivores may cause chronic mortality through predisposition to fungal pathogens or above-ground herbivores (Whitney, 1961; Hunter, 2001; Zhu *et al.*, 2008) or the insects may girdle and kill young trees. Notable examples include weevils such as *Hylastes ater* (F.) (Coleoptera: Curculionidae: Scolytinae) and *Hylobius abietis* (L.) (Coleoptera: Curculionidae) in Europe (Eidmann, 1997; Leahy *et al.*, 2007).

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In North America, an emerging concern in forest management is the Warren root collar weevil *Hylobius warreni* Wood (Coleoptera: Curculionidae), a native, flightless insect distributed transcontinentally throughout northern coniferous forests (Cerezke, 1994).

Warren root collar weevils live for up to 5 years and feed on all life stages of host trees (Cerezke, 1994). Females lay up to 25 eggs per year on the root collars. Larval development normally spans a 2-year period. Upon eclosion, larvae bore into and feed on the bark and cambium. Trees produce excessive amounts of pitch, which larvae mix with frass to form protective coverings. During larval feeding, wound accumulation predisposes trees to fungal infection and reduces stem growth. In young trees, larval girdling frequently results in mortality (Grant, 1966; Cerezke, 1994). For example, mortality rates in stands of lodgepole pine *Pinus contorta* Douglas var. *latifolia* (Engelmann) aged <30 years are historically in the range 5–10% in western Canada (Cerezke, 1994). Recently, much higher mortality rates have been reported. Schroff *et al.* (2006), for example, found up to 16.6% mortality in regenerating lodgepole pine stands. Because of the large-scale outbreak of the mountain pine beetle *Dendroctonus ponderosae* Hopkins, in British Columbia (Aukema *et al.*, 2006), and the resulting increase in reforestation activities, there is growing concern regarding the effects of *H. warreni*-induced mortality on replanted seedlings, given the reduced host-pool of mature pine (Klingenberg, 2008).

Adult oviposition takes place during the summer months, between May and August, peaking in early July. Prior to oviposition, weevils feed nocturnally. They feed primarily on the upper surface of branches and some terminal buds, often in the upper part of the tree. Warren root collar weevils feed on most pine and spruce species in Canada (Cerezke, 1994). In western Canada, reported hosts include lodgepole pine; white spruce *Picea glauca* Moench; western white pine *Pinus monticola* Douglas ex D. Don; Engelmann spruce *Picea engelmannii* Parry ex. Engelmann; Douglas-fir *Pseudotsuga menziesii* Mirbel; and sub-alpine fir *Abies lasiocarpa* (Hook.) Nutt. (Grant, 1966; Cerezke, 1994).

Although a large number of coniferous hosts have been reported, information on the insects' relative feeding preferences among host trees is lacking. This may be a result, in part, of the relative difficulty in obtaining insects for bioassays. Adults are primarily nocturnal and, to date, any potential chemical attractants remain unknown. Recent development of a trap that exploits the insects' feeding behaviour, however, has improved the acquisition of test specimens (Klingenberg *et al.*, 2009; Björklund, 2009) compared with previous methods (Cerezke, 1994). Information on feeding preferences among different hosts is important for developing appropriate silvicultural recommendations, developing host inventories for trade records as wood product and insect movement increases, and deriving new antifeedant control strategies, as has been proposed for *H. abietis* with deciduous trees (Leather *et al.*, 1994; Månsson *et al.*, 2005).

The present study aimed to investigate feeding and ovipositional preferences of adult *H. warreni* on the bark of small branches in a series of choice and no-choice bioassays in a laboratory setting. We chose three coniferous hosts: lodgepole

pine, interior hybrid spruce *Picea glauca* × *engelmannii*, and Douglas-fir. These species represent the most economically important trees in western Canada. A fourth tree, trembling aspen *Populus tremuloides* Michx, was also tested. Field observations have indicated substantial mortality to juvenile pine by Warren root collar weevil in areas with high aspen concentrations, leading to the hypothesis that insects may be using aspen as a facultative host.

Materials and methods

Experimental insects

Insects were collected in early summer each year from an approximately 10–20-year-old stand of lodgepole pine near Prince George, British Columbia, Canada (53°55'N, 122°49'W), using a newly-developed 'Björklund' funnel trap that is attached to the base of trees (Klingenberg *et al.*, 2009; Björklund, 2009). Briefly, the trap permits the insects to climb the tree into the foliage to feed nocturnally but captures them at the base of the tree when they descend to rest. The trap uses no chemicals, and insects are captured alive. Upon collection, the weevils were retained in plastic holding containers (height 140 mm, width 110 mm) in groups of ten insects per container with a damp paper towel, and were fed small branches of lodgepole pine daily. The containers were kept in a laboratory fridge at 7°C to slow down the insects' metabolism (Toivonen & Viiri, 2006). Insects were stored for a maximum of 6 weeks prior to assays, and survival during trapping, collection and storage was almost 100%. Weevils were starved before the commencement of bioassays (Månsson & Schlyter, 2004; Toivonen & Viiri, 2006) for a period of 48 h in the fridge at 7°C.

Host plants

Branches of lodgepole pine, interior hybrid spruce, Douglas-fir and aspen were cut from mature trees in a natural forest adjacent to the campus of the University of Northern British Columbia, Prince George, British Columbia, Canada. Only nonbranching, single-lateral branches were used, visibly free from insect feeding and pathogens and, in all cases, the needles and/or leaves were retained. Cuttings were approximately 50 mm in length with a diameter (i.e. thickness) of 1–3 mm for the choice bioassays, and 100 mm in length and 1–3 mm in diameter for the no-choice experiment. Branch lengths were measured with a standard ruler with increments of 1 mm, and branch diameters were measured using an electronic digital caliper with a range 0–150 mm, averaging three measurements along the length of the branch (Örlander *et al.*, 2000; Örlander *et al.*, 2001). In all experiments, branches were used immediately upon harvesting.

To minimize spatial variation, and because these insects are flightless and presumably do not move great distances in mature forests (Cerezke, 1994; Klingenberg *et al.*, 2009), branches were harvested from trees restricted to a small area of the forest in 2007. This protocol restricted sampling to low numbers of spruce in 2007. Accordingly, additional feeding assays were performed in 2008 using foliage from individual trees over a

greater area. In instances where branches had been taken from the same tree in 2007, the data were treated as subsamples.

Choice assays

Four branch sections, one from each tree species, were placed on a piece of filter paper (width 140 mm) in a square plastic container (height 50 mm, width 140 mm wide). The filter paper was moistened with 0.8 mL of distilled water to maintain humidity inside this arena (Raffa *et al.*, 2002; Toivonen & Viiri, 2006; Leahy *et al.*, 2007). One adult weevil, starved for 48 h previously, was placed inside the container, and allowed to feed on the four branches. Once a day, each branch of each species was removed from the container and the surface area of bark consumed was estimated by holding a flexible grid (mm²) on a sheet of ethyl acetate transparent paper over the feeding scars on each branch section (Örlander *et al.*, 2000; Bratt *et al.*, 2001; Örlander *et al.*, 2001; Månsson & Schlyter, 2004; Nordlander *et al.*, 2005). Branches were returned immediately to each container after measuring. Individual feeding scar areas were summed for each species to yield a total area of bark consumed by the insect. The number of eggs was not counted because it is impossible to correlate feeding on specific hosts with oviposition in choice assays. Conducted over periods of 6 days, the choice assay was replicated 13 times from 11–16 July 2007, and six times from 27 July to 1 August 2008, under an LD 17:7 h photoperiod (lights on 05.00 h) at 21 °C and 20% relative humidity. After the bioassays, the sex of each insect was determined by examining differences in the anatomy of the eighth sternite, the arrangement of setae on the last sternite, and the presence and shape of the apodemes in females via dissection (Warren, 1960; Öhrn *et al.*, 2008).

No-choice assays

One branch of each species was placed on a piece of filter paper in a container with a single test insect. The filter paper was moistened with 0.8 mL of distilled water when necessary. Similar to the choice assays, each branch section was removed daily to calculate the surface area of bark consumed by each insect and returned to the container. Oviposition was measured by counting the number of eggs laid each day. Oviposition was quite indiscriminate on both foliage and filter paper and eggs were sufficiently large to be visually apparent (see Results). Thus, it was not necessary to destructively peel individual branches when searching for eggs. Feeding trials were replicated 14, 9, 18 and 14 times for the pine, spruce, Douglas-fir and aspen, respectively, for a total of 55 bioassays over the 2 years. The assays were conducted over the same time periods in a room with controlled environmental conditions matching those previously described. Similar to the choice assays, the sex of each insect was determined after the bioassays.

Statistical analysis

For the choice assays, the occurrence of feeding, surface area consumed, feeding rate, and number of feeding days were

analyzed separately by a mixed-effects analysis of variance (ANOVA), where the species of tree was the fixed effect of interest, and experimental container was incorporated as a random effect. For the no-choice assays, the occurrence of feeding, surface area consumed, feeding rate and number of feeding days were analyzed, as well as the number of fecal pellets and number of eggs laid. Again, each response variable was examined in ANOVA models, where the species of tree was the experimental factor. A term for year was included in all ANOVAs to control for potential year-to-year variation. In exploratory analysis for the no-choice assays, we also initially incorporated the gender of the test insect as a term in the models. This term was subsequently removed, however, because gender did not affect any of the response variables examined (see Results).

A variance components analysis was performed with spruce data from the no-choice assays to compare the relative inter-branch variation with inter-tree variation in total bark consumption. Variation attributed to branches within trees and variation between spruce trees was measured from the standard deviations of the respective random effects in a linear model fitting mean surface area consumed to an intercept. The standard deviations of each component were squared to obtain the relative variances attributable to inter-branch and inter-tree effects.

In all analyses, response variables were square root-transformed as necessary to reduce heteroscedasticity, except for measurements on the presence/absence of feeding. These binomial responses were analyzed in a logistic linear model framework. Where significant treatment effects occurred ($\alpha = 0.05$), pairwise comparisons were performed with Tukey's procedure. All data analyses were performed in R, version 2.6.1, using software packages MASS, version 7.2-38, LME4, version 0.99875-9, and MULTCOMP, version 1.0-0 (Ihaka & Gentleman, 1996; R Development Core Team, 2008).

Results

Choice assays

The prevalence of feeding by the weevils differed significantly among tree species ($F_{3,54} = 5.30$; $P = 0.0028$). Pine was the preferred choice of food, with $73.79 \pm 10.4\%$ of the insects feeding. The next preferred host was Douglas-fir ($52.6 \pm 11.8\%$), followed by spruce ($26.3 \pm 10.4\%$). In the presence of coniferous branches, aspen was only eaten by one insect. Despite fewer weevils feeding on Douglas-fir, weevils consumed almost equivalent amounts of Douglas-fir and pine, 54 ± 11 and 50 ± 16 mm², respectively (Fig. 1A). Weevils consumed significantly less spruce (10 ± 6 mm²) and aspen (<1 mm²). Weevils only consumed bark when feeding, and did not appear to feed on foliage.

A variety of feeding metrics is presented in Table 1. In general, weevils did not consume bark every night but would feed and then rest. The number of days that the insects fed on each branch differed significantly by tree species (Table 1). On average, pine was fed upon approximately two of the 6 days, followed by Douglas-fir (1.8 ± 0.5 days) and then spruce (0.7 ± 0.4 days). The number of days that weevils fed on pine versus Douglas-fir was not significantly different

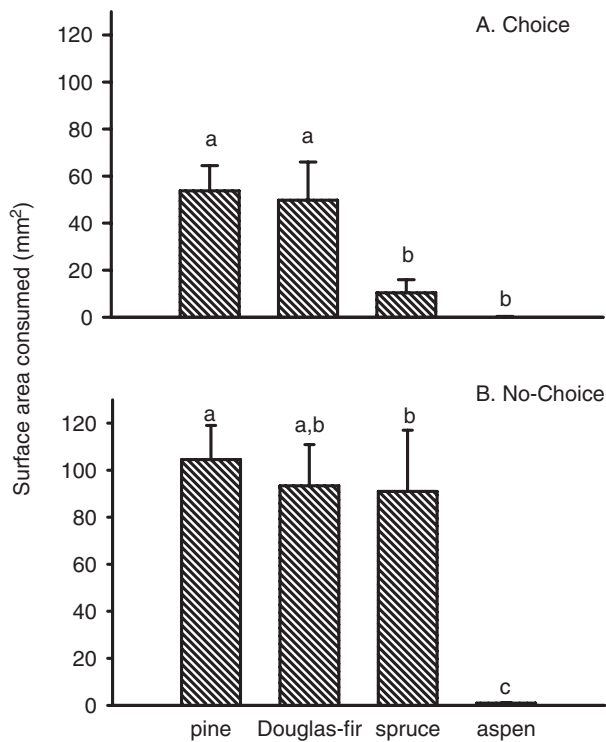


Figure 1 Mean \pm SE surface area of bark consumed on branches of the four tree species by adult *Hylobius warreni*. Different letters above the columns indicate significant treatment differences (Tukey's procedure). (A) Choice feeding bioassay ($F_{3,54} = 11.54$, $P < 0.0001$). (B) No-choice bioassay ($F_{3,50} = 20.13$, $P < 0.0001$).

but pine was fed upon more often than spruce. The average daily rate of bark consumed over the 6-day period differed significantly by tree species (Table 1), with the greatest average rate of consumption occurring on Douglas-fir and pine (9.1 and 8.4 mm²/day, respectively). On average, the insects ate only 1.7 mm² of spruce bark per day when presented with options of the other two conifers. Gender and year did not affect feeding amount or preference for any of the variables measured ($P > 0.05$). Fourteen of the 19 insects were females.

Table 1 Measurements of feeding and ovipositional performance on Warren root collar weevil in laboratory bioassays on the bark of conifer and nonconifer hosts

Measurement	Pine	Douglas-fir	Spruce	Aspen	F^a	P
Choice bioassay						
Number of days with feeding	2.4 \pm 0.4 ^a	1.8 \pm 0.5 ^{ab}	0.7 \pm 0.4 ^{bc}	0.1 \pm 0.1 ^c	10.63	<0.0001
Mean bark area consumed/day	9.1 \pm 1.8 ^a	8.4 \pm 2.7 ^a	1.7 \pm 0.9 ^b	0.03 \pm 0.03 ^b	20.16	<0.0001
No-choice bioassay						
Number of days with feeding	4.0 \pm 0.4 ^a	3.1 \pm 0.5 ^a	2.9 \pm 0.7 ^a	0.4 \pm 0.3 ^b	15.68	<0.0001
Mean bark area consumed/day	17.5 \pm 2.4 ^a	15.6 \pm 2.9 ^{ab}	15.1 \pm 4.3 ^b	0.2 \pm 0.1 ^c	20.16	<0.0001
Number of eggs laid	2.1 \pm 0.4	3.8 \pm 0.6	2.8 \pm 1.0	2.6 \pm 0.6	1.02	0.3954

^aDegrees of freedom: 3,54 for choice bioassays and 3,50 for no-choice bioassays, except for the number of eggs, where d.f. = 3,37 (male insects are excluded from analysis).

Values under each host type are the mean \pm SE. Means followed by the same superscript letter across each row are not significantly different (Tukey's procedure). Choice assay, $n = 19$; No-choice assay, $n = 55$.

No-choice assays

Similar to the choice bioassay, the prevalence of feeding by the weevils differed significantly among tree species ($\chi^2 = 27.27$, d.f. = 3, $P < 0.0001$). All insects fed on pine, whereas 83 \pm 9% fed on Douglas-fir and 71 \pm 15% on spruce. Although only one insect fed on aspen in the presence of coniferous branch sections, three out of 14 insects nibbled on aspen in the no-choice bioassay. This feeding was very limited (0.9 \pm 0.7 mm²). Insects consumed the greatest amount of bark on pine (105 \pm 15 mm²), followed by Douglas-fir (93 \pm 18 mm²) and spruce (91 \pm 26 mm²) (Fig. 1B).

Variation in spruce consumption was greater between trees than between branches in a single tree. Inter-tree variance was estimated to be 21.4 mm. This was almost twice the estimated variance in feeding activity between branches (12.4 mm).

Weevils did not consume bark each night but would feed and then rest. Similar to the choice bioassay, the number of days that feeding occurred on each branch significantly differed by tree species (Table 1). Feeding occurred most often on pine (4.0 \pm 0.4 days), followed by Douglas-fir (3.1 \pm 0.5 days), spruce (2.9 \pm 0.7 days) and aspen (0.4 \pm 0.3 days). The number of days weevils exhibited feeding activity was not significantly different among the conifers.

Similar to the choice bioassay, the average rate of bark consumption by the weevils differed significantly by tree species (Table 1). Weevils consumed pine and Douglas-fir at the highest rates (17.5 \pm 2.4 and 15.6 \pm 2.9 mm²/day, respectively). The rate on pine was significantly higher than spruce, which approached the rate on Douglas-fir at 15.1 \pm 4.3 mm²/day.

Forty-two of the insects in the no-choice experiment were females. Similar to the choice experiment, gender did not affect any of the feeding metrics examined ($P > 0.05$ for all variables). All but six females oviposited, laying a mean \pm SD of 3.1 \pm 1.4 eggs. The number of eggs did not vary with the species of tree upon which the insects were feeding ($F_{3,37} = 1.02$, $P = 0.40$) and the number of eggs did not vary with total bark consumed ($F_{1,39} = 0.24$, $P = 0.62$). Eggs were laid indiscriminately, on both the branches and the surrounding filter paper in the containers. No eggs were inserted into the bark. The eggs were an elliptical shape (approximate length 1.8 mm and width 1.3 mm), with a creamy-white colour.

Discussion

The findings obtained in the present study indicate that the occurrence of feeding, the surface area of bark consumed, the number of days of feeding and the rate of feeding by *H. warreni* are all greatest on lodgepole pine and Douglas-fir, followed by interior hybrid spruce. These apparent host preferences do not necessarily reflect field collections or records of larval activity. For example, Grant (1966) found more weevils on spruce than on lodgepole pine, citing Douglas-fir as an occasional host. Similarly, Cerezke (1994) does not list Douglas-fir as a host in western Canada but includes it as a 'rare host' in eastern Canada. Douglas-fir is a common tree in western Canada, and is found on dry sites. In our choice and no-choice bioassays, female insects did not discriminate among hosts when ovipositing. We note, however, that our insects were limited to small, clipped branches, without access to root collars and precluding additional cues that are potentially important in oviposition and host selection among *Hylobius* spp., such as induced defensive chemicals in the resin of host trees, visual silhouettes, soil type and substrate topography (Cerezke, 1994; Björklund *et al.*, 2003; Björklund *et al.*, 2005; Nordlander *et al.*, 2005; Robert & Lindgren, 2006). Nonetheless, the indiscriminate nature of oviposition in both choice and no-choice assays suggests that differences in insect population density among conifer species in natural settings may be primarily the result of variation in larval survival and performance on different hosts, rather than differences in ovipositional preferences by adult females.

Currently, there is a landscape-level outbreak of *D. ponderosae* in western Canada covering an area approximately 14 million ha in size. Salvage operations are creating landscape mosaics where young stands reforested with lodgepole pine, highly susceptible to *H. warreni*, frequently exist adjacent to mature, unsalvaged stands with high tree mortality from *D. ponderosae*. Although adult *H. warreni* are flightless, they migrate readily from areas with dead trees into habitats with green trees (Klingenberg *et al.*, 2009). Hence, there is increasing mortality to young trees in these replantings of lodgepole pine (Klingenberg, 2008). The findings obtained in the present study indicating that spruce is less palatable than pine, in concert with the fact that mortality of lodgepole pine from larval feeding is greater on planted versus naturally regenerating stock (Robert & Lindgren, 2006), suggest that increased mixes of spruce may be beneficial in reforestation activities where high weevil populations are likely to occur (Cerezke, 1994). It is possible, for example, that planting buffer strips of spruce or nonhosts such as aspen may reduce weevil ingress into areas reforested with lodgepole pine. At the same time, if feeding preferences of adult insects do not reflect ovipositional strategies, *H. warreni* may be most successful in stands of high species diversity that provide hosts for both larvae and adults. Hence, silvicultural strategies of planting lodgepole pine surrounded by a mix of tree species may warrant further examination in high-risk sites.

We did not find strong evidence of phagostimulants or antifeedants in our assays, although there was substantial variation in palatability between spruce trees (i.e. almost double that of within-tree variation). All spruce were interior hybrid spruce, a naturally occurring hybrid between *P. glauca* and *P. engelmannii*, and the most common type of spruce in central British Columbia. Our finding that certain spruce individuals

were highly unpalatable suggests that some conifer bark may have contained compound(s) that deter feeding (Bratt *et al.*, 2001). Plant-derived antifeedants or feeding depressants against *H. abietis* are well known, some of which act even in the presence of suitable coniferous hosts (Leather *et al.*, 1994; Lindgren *et al.*, 1996; Klepzig & Schlyter, 1999; Sunnerheim *et al.*, 2007). For example, Månsson and Schlyter (2004) reported that the bark of nonhost plants may exhibit antifeedant properties, causing the insects to decline food in choice tests even when both host and nonhost plants are present. This effect may not be ubiquitous, however, because Löf *et al.* (2004) found normal levels of feeding on spruce, even in the presence of broad-leafed plants. These findings concur with the results obtained in the present study because the presence of aspen twigs did not prevent or inhibit feeding on conifers in the choice assays. Although no antifeedant properties were evident in aspen, we note that this species was never consumed in the choice bioassay, and was consumed only in very small amounts in the no-choice bioassay. The minimal feeding suggests that *H. warreni* do not use trembling aspen as a facultative host in natural settings. Thus, high weevil damage on young conifers near pockets of aspen are likely to be a result of the concentration of weevils on the suitable coniferous hosts.

The nature of unpalatability of trembling aspen and a diversity of other nonhost plants should be screened in future studies before excluding the possibility of bioactive antifeedant compounds for *H. warreni* because structure–activity relationships show promise in a related *Hylobius* species (Sunnerheim *et al.*, 2007; Bohman *et al.*, 2008). We note, however, that the number of choices presented to an organism in a choice bioassay affects the strength of results because there is a limit to the number of choices that a test subject can effectively make (Raffa *et al.*, 2002). Four-way choices have traditionally offered only somewhat statistically significant results, and it appears that the fewer the choices, and the greater number of replicates, the stronger the significance of the results (Raffa *et al.*, 2002).

Male and female weevils consumed similar amounts of bark in our bioassays. By contrast, previous studies on other weevil species have found that females eat significantly more than males (Bylund *et al.*, 2004; Wainhouse *et al.*, 2004; Pinski *et al.*, 2005), possibly because of the positive relationship between feeding and realized fecundity (Bylund *et al.*, 2004). In *H. abietis*, female diet affects fecundity (Wainhouse *et al.*, 2001). Although the present study did not reveal a significant relationship between diet type and oviposition by *H. warreni*, we cannot infer that a relationship does not exist because all insects were fed a similar diet and were most likely gravid prior to the start of the experiment. Moreover, reproductive development is linked to nutritional quality, such as nitrogen concentration, which undoubtedly varies across hosts (Wainhouse *et al.*, 2004).

Each of the performance metrics has advantages and limitations in our choice and no-choice assays. Measuring the amount of surface area of bark consumed, although comprising a standard metric, may not account for differences in bark chemistry, texture and thickness between species (Månsson & Schlyter, 2004; Pinski *et al.*, 2005). Moreover, it may not capture behavioural differences among insects exhibiting disparate feeding rates because the total surface area consumed would

appear to be the same for insects that feed a small amount each day versus those that feed voraciously only once per week. In our assays, insects exhibited similar feeding frequencies on conifers but only nibbled on the unpalatable aspen. Hence, for the purposes of the present study, the total surface area consumed appears to be a metric robust to the feeding behaviours exhibited by Warren root collar weevil. All feeding metrics may be affected to some degree by artefacts of experimental design, such as insect collections and maintenance feedings standardized on one type of host prior to experiment initiation, or pre-experiment starvation creating excessive feeding behaviour on otherwise unpalatable hosts shortly after commencing bioassays.

In summary, in the present study, we have demonstrated that pine and Douglas-fir are preferred to spruce or a nonhost such as aspen in feeding assays of *H. warreni* using cut branches in a laboratory environment. The results obtained have implications to the management of this insect, especially in high-risk areas such as reforested areas adjacent to mature stands with reduced host pools from *D. ponderosa* activity on the landscape of western Canada. To potentially bring new recommendations for the management of *H. warreni* using silvicultural strategies to fruition, additional research is needed to determine whether the preferences noted in the present study persist in the presence of induced defensive chemistry of live trees and, similarly, whether the indiscriminate ovipositional strategies noted in the laboratory reflect behaviours among root collars in field settings. Additionally, investigating the rates of increase on various hosts and determining the movement patterns through habitats comprised of various hosts are both subjects that warrant additional research (Klingenberg *et al.*, 2009).

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References

- Aukema, B.H., Carroll, A.L., Zhu, J., Raffa, K.F., Sickley, T.A. & Taylor, S.W. (2006) Landscape level analysis of mountain pine beetle in British Columbia, Canada: spatiotemporal development and spatial synchrony within the present outbreak. *Ecography*, **29**, 427–441.
- Björklund, N. (2009) Non-destructive tree-trunk funnel trap for capturing *Hylobius warreni* (Coleoptera: Curculionidae) ascending stems of trees. *The Canadian Entomologist*, **141**, 422–424.
- Björklund, N., Nordlander, G. & Bylund, H. (2003) Host-plant acceptance on mineral soil and humus by the pine weevil *Hylobius abietis* (L.). *Agricultural and Forest Entomology*, **5**, 61–65.
- Björklund, N., Nordlander, G. & Bylund, H. (2005) Olfactory and visual stimuli used in orientation to conifer seedlings by the pine weevil, *Hylobius abietis*. *Physiological Entomology*, **30**, 225–231.
- Bohman, B., Nordlander, G., Nordenhem, H., Sunnerheim, K., Borg-Karlson, A.-K. & Unelius, C.R. (2008) Structure-activity relationships of phenylpropanoids as antifeedants for the pine weevil *Hylobius abietis*. *Journal of Chemical Ecology*, **34**, 339–352.
- Bratt, K., Sunnerheim, K., Nordenhem, H., Nordlander, G. & Langström, B. (2001) Pine Weevil (*Hylobius abietis*) antifeedants from lodgepole pine (*Pinus contorta*). *Journal of Chemical Ecology*, **27**, 2253–2261.
- Bylund, H., Nordlander, G. & Nordenhem, H. (2004) Feeding and oviposition rates in the pine weevil *Hylobius abietis* (Coleoptera: Curculionidae). *Bulletin of Entomological Research*, **94**, 307–317.
- Cerezke, H.F. (1994) Warren Rootcollar Weevil, *Hylobius warreni* Wood (Coleoptera: Curculionidae) in Canada: ecology, behaviour, damage relationships, and management. *The Canadian Entomologist*, **126**, 1383–1442.
- Eidmann, H.H. (1997) Assessment of pine weevil numbers on clear-cut and forest sites with shelter boards and pitfall traps. *Anzeiger Fur Schadlingskunde Pflanzenschutz Umweltschutz*, **70**, 68–72.
- Grant, J. (1966) The hosts and distribution of the root weevils *Hylobius pinicola* (Couper) and *H. warreni* Wood in British Columbia. *Journal of the Entomological Society of British Columbia*, **63**, 3–5.
- Hunter, M.D. (2001) Out of sight, out of mind: the impacts of root-feeding insects in natural and managed systems. *Agricultural and Forest Entomology*, **3**, 3–9.
- Ihaka, I. & Gentleman, R. (1996) R: a language for data analysis and graphics. *Journal of Computational and Graphical Statistics*, **5**, 299–314.
- Klepzig, K.D. & Schlyter, F. (1999) Laboratory evaluation of plant-derived antifeedants against the pine weevil *Hylobius abietis* (Coleoptera: Curculionidae). *Forest Entomology*, **92**, 644–650.
- Klingenberg, M.D. (2008) *Spatiotemporal associations between forests impacted by mountain pine beetle and adjacent replantings impacted by Warren root collar weevil*. M.Sc. Thesis, Natural Resources and Environmental Science, University of Northern British Columbia, Prince George, Canada, 88 pp.
- Klingenberg, M.D., Björklund, N. & Aukema, B.H. (2009) Seeing the forest through the trees: differential dispersal of *Hylobius warreni* Wood within modified forest habitats. *Environmental Entomology*, in press.
- Leahy, M.J.A., Oliver, T.H. & Leather, S.R. (2007) Feeding behaviour of the black pine beetle *Hylastes ater* (Coleoptera: Scolytidae). *Agricultural and Forest Entomology*, **9**, 115–124.
- Leather, S.R., Ahmed, S.I. & Hoyal, L. (1994) Adult feeding preferences of the large pine weevil, *Hylobius abietis* (Coleoptera, Curculionidae). *European Journal of Entomology*, **91**, 385–389.
- Lindgren, B.S., Nordlander, G. & Birgersson, G. (1996) Feeding deterrence of verbenone to the pine weevil, *Hylobius abietis* (L.) (Col., Curculionidae). *Journal of Applied Entomology*, **120**, 397–403.
- Löf, M., Isacson, G., Rydberg, D. & Welander, T. (2004) Herbivory by the pine weevil (*Hylobius abietis* L.) and short-snouted weevils (*Strophosoma melanogrammum* Forst. and *Otiorhynchus scaber* L.) during the conversion of a wind-thrown Norway spruce forest into a mixed-species plantation. *Forest Ecology and Management*, **190**, 281–290.
- Månsson, P.E. & Schlyter, F. (2004) *Hylobius* pine weevils adult host selection and antifeedants: feeding behaviour on host and non-host woody Scandinavian plants. *Agricultural and Forest Entomology*, **6**, 165–171.

- Månsson, P.E., Eriksson, C. & Sjödin, K. (2005) Antifeedants against *Hylobius abietis* pine weevils: an active compound in extract of bark of *Tilia cordata* Linden. *Journal of Chemical Ecology*, **31**, 989–1001.
- Nordlander, G., Bylund, H. & Björklund, N. (2005) Soil type and microtopography influencing feeding above and below ground by the pine weevil *Hylobius abietis*. *Agricultural and Forest Entomology*, **7**, 107–113.
- Öhrn, P., Klingenberg, M.D., Hopkins, G.R. & Björklund, N. (2008) Two non-destructive techniques for determining the sex of live adult *Hylobius warreni*. *The Canadian Entomologist*, **140**, 617–620.
- Örlander, G., Nordlander, G., Wallertz, K. & Nordenhem, H. (2000) Feeding in the crowns of Scots pine trees by the pine weevil *Hylobius abietis*. *Scandinavian Journal of Forest Research*, **15**, 194–201.
- Örlander, G., Nordlander, G. & Wallertz, K. (2001) Extra food supply decreases damage by the pine weevil *Hylobius abietis*. *Scandinavian Journal of Forest Research*, **16**, 450–454.
- Pinski, R.A., Mattson, W.J. & Raffa, K.F. (2005) Host breadth and ovipositional behavior of adult *Polydrusus sericeus* and *Phyllobius oblongus* (Coleoptera: Curculionidae), nonindigenous inhabitants of northern hardwood forests. *Environmental Entomology*, **34**, 148–157.
- Raffa, K.F., Havill, N.P. & Nordheim, E.V. (2002) How many choices can your test animal compare effectively? Evaluating a critical assumption of behavioural preference tests. *Oecologia*, **133**, 422–429.
- R Development Core Team (2008) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Austria, <http://www.R-project.org>, 2008.
- Robert, J.A. & Lindgren, B.S. (2006) Relationships between root form and growth, stability, and mortality in planted versus naturally regenerated lodgepole pine in north-central British Columbia. *Canadian Journal of Forest Research*, **36**, 2642–2653.
- Schroff, A.Z., Lindgren, B.S. & Gillingham, M.P. (2006) Random acts of weevil: a spatial analysis of *Hylobius warreni* attack on *Pinus contorta* var. *latifolia* in the sub-boreal spruce zone of northern British Columbia. *Forest Ecology and Management*, **227**, 42–49.
- Sunnerheim, K., Nordqvist, A., Nordlander, G. *et al.* (2007) Quantitative structure–activity relationships of pine weevil antifeedants, a multivariate approach. *Journal of Agricultural and Food Chemistry*, **55**, 9365–9372.
- Toivonen, R. & Viiri, H. (2006) Adult large pine weevils *Hylobius abietis* feed on silver birch *Betula pendula* even in the presence of conifer seedlings. *Agricultural and Forest Entomology*, **8**, 121–128.
- Wainhouse, D., Ashburner, R. & Boswell, R. (2001) Reproductive development and maternal effects in the pine weevil *Hylobius abietis*. *Ecological Entomology*, **26**, 655–661.
- Wainhouse, D., Boswell, R. & Ashburner, R. (2004) Maturation feeding and reproductive development in adult pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae). *Bulletin of Entomological Research*, **94**, 81–87.
- Warren, G.L. (1960) External anatomy of the adult of *Hylobius warreni* Wood (Coleoptera: Curculionidae) and comparison with *H. pinicola* (Couper). *The Canadian Entomologist*, **92**, 321–341.
- Whitney, R.D. (1961) Root wounds and associated root rots of white spruce. *Forestry Chronicle*, **37**, 401–411.
- Zhu, J., Rasmussen, J.G., Moller, J.M., Aukema, B.H. & Raffa, K.F. (2008) Spatial-temporal modeling of forest gaps generated by colonization from below-and above-ground bark beetle species. *Journal of the American Statistical Association*, **103**, 162–177.

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