

The role of vision in the host orientation behaviour of *Hylobius warreni*

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- Abstract**
- 1 Visual stimuli, often in combination with olfactory stimuli, are frequently important components of host selection by forest-dwelling phytophagous insects.
 - 2 Warren root collar weevil *Hylobius warreni* Wood (Coleoptera: Curculionidae) is a native insect in western Canada, where larvae feed primarily on lodgepole pine *Pinus contorta* and can girdle and kill young trees. This weevil is an emerging problem in areas heavily impacted by mountain pine beetle *Dendroctonus ponderosae* Hopkins.
 - 3 No olfactory attractants have been identified for this insect, making monitoring and management difficult. Thus, we investigated the role of vision in the host-finding behaviour of Warren root collar weevil in the absence of known olfactory cues.
 - 4 We conducted three experiments in field enclosure plots aiming to characterize aspects of host-finding behaviour by adult Warren root collar weevil.
 - 5 We found that both male and female weevils were readily attracted to vertical plastic silhouettes in the shape of a trunk, crown or tree at distances of less than 4 m. This pattern of attraction persisted over 2 years in two slightly different study designs. Blinding the insects removed their ability to orient to these silhouettes, indicating that host-finding behaviour has a strong visual component. The use of different colour trunks and crowns (black, white and green) did not change the patterns of attraction of the insects to the silhouettes.
 - 6 Exploiting visual attraction in this walking insect may present a new management tool in forest protection strategies.

Keywords Host selection, host-finding behaviour, silhouettes, vision, Warren root collar weevil.

Introduction

Host selection by phytophagous insects is characterized by two activities: host location and host assessment (Dethier, 1983). Locating hosts requires finding appropriate habitat and then identifying a host plant, potentially among other nonhost vegetation (Dethier, 1983; Huber *et al.*, 2000; Raffa, 2001; Bernays, 2003). Insects utilize various strategies to maximize their chances of encountering an appropriate plant, such as increasing activity, moving randomly, turning frequently and/or responding to various host stimuli with an orderly sequence of behaviour patterns (Dethier, 1983). Olfactory and visual stimuli are both perceived as attractive cues by many insects when

initially locating plants on which to feed and oviposit (Bernays & Chapman, 1994; Bernays, 2003).

Although both types of cues have been shown to be integrated by phytophagous insects, including many types of forest-dwelling arthropods (Borden *et al.*, 1985; Strom *et al.*, 1999, 2001; Björklund *et al.*, 2005; Campbell & Borden, 2006a, b), olfactory cues are frequently viewed as the more important. Not unexpectedly, the role of olfaction has received the most attention in studies of how insects select their hosts. The use of visual stimuli in detecting plants has been reported for several species (Prokopy & Owens, 1983; Reeves, 2011), although few insects were found to use visual cues in the absence of olfactory cues (Stenberg & Ericson, 2007; Reeves *et al.*, 2009; Reeves, 2011).

Warren root collar weevil *Hylobius warreni* Wood (Coleoptera: Curculionidae) is a phytophagous insect that is

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native throughout Canada's boreal forests (Cerezke, 1994). Adult weevils can reach 5 years of age. The weevils cannot fly, and thus they encounter hosts as they walk along the forest floor (Grant, 1966; Cerezke, 1994). In British Columbia, Canada, the weevil's primary host is lodgepole pine *Pinus contorta* var. *latifolia* (Warren, 1956; Wood, 1957; Grant, 1966). Adults ascend conifer trees at dusk to feed on the branches, bark and needles. Feeding by adult Warren root collar weevil typically does not cause tree mortality (Warren, 1956; Warner, 1966). Larval feeding, however, may cut into the cambial and xylem tissues of the host's large lateral roots or root collar (Warren, 1956; Cerezke, 1994). In young trees, complete girdling can be accomplished by as few as one to three larvae. In trees with larger diameters, many more weevils are required to complete girdling, although insects rarely occur at such high densities. Warren root collar weevils can cause mortality in stands as old as 30 years of age, although peak mortality occurs in stands aged 5–10 years of age (Cerezke, 1994).

The current epidemic of mountain pine beetle *Dendroctonus ponderosae* Hopkins has killed over 630 million m³ of mature lodgepole pine in British Columbia and Alberta since 1998 and is predicted to kill approximately 67% of mature pine in British Columbia by 2016 (Aukema *et al.*, 2006; Walton, 2010). As a result, salvage logging and reforestation has begun to shift the age structure and species composition of large areas of forest. Recently, studies have reported increased mortality to young trees caused by Warren root collar weevil (Robert & Lindgren, 2006), especially in areas where harvested and reforested lands are located adjacent to stands of mature lodgepole pine heavily affected by mountain pine beetle where the timber has not been removed. Such patterns are consistent with the hypothesis that weevils are migrating from forests with depleted host pools to replanted areas in search of new hosts (Klingenberg *et al.*, 2010b).

Despite the increased importance of the insect, little is known about the host selection mechanisms of Warren root collar weevil. In closely-related species of *Hylobius*, volatiles of host terpenes and ethanol play prominent roles in host attraction (Tilles *et al.*, 1986a, b; Raffa & Hunt, 1988). Attraction to such compounds has been exploited to increase trapping efficiency and has greatly aided the development of management plans for pine weevils (Nordlander, 1987; Raffa & Hunt, 1988; Hunt & Raffa, 1989; Rieske & Raffa, 1993). Studies aiming to determine whether Warren root collar weevils are similarly attracted to host volatiles have yielded inconclusive results (K. Sambaraju and B. S. Lindgren, personal communication; Duke & Lindgren, 2006). In these field and laboratory olfactometer studies, weevils failed to show responses to chemical stimuli such as monoterpene components that characterize lodgepole pine. Moreover, no clear links have been found between tree chemistry and susceptibility to attack by Warren root collar weevil (Duke & Lindgren, 2006).

It is possible that vision plays a prominent role in host orientation for Warren root collar weevil. In a review on the ecology, behaviour and management of the weevil, Cerezke (1994) commented that host selection was probably visual in nature and thus influenced by silhouettes. This hypothesis was proposed after noticing that the capture frequency of

adult weevils was positively correlated with an increasing tree diameter (Cerezke, 1994). Moreover, a number of studies have observed that weevils orient and move toward two-dimensional silhouettes in laboratory settings (Hoover, 2000; Horning & Lindgren, 2002). These findings are consistent with those found for other conifer-feeding insects, including other pine weevil species that integrate visual cues in the presence of olfactory cues. For example, the pales weevil *H. pales* Herbst is more attracted to traps baited with ethanol and turpentine that have white silhouettes than to traps with black or green silhouettes (Hunt & Raffa, 1991).

The present study aimed to provide additional information concerning the role of vision in the host-finding behavior of Warren root collar weevil. We investigated the hypothesis that vision plays a role in initial steps of the weevils' host finding by exploring three questions. First, are weevils attracted to visual cues? If so, does blinding the insects remove their host-finding ability? Finally, does colour affect locomotor response to vertical silhouettes?

Materials and methods

Site set-up

During the summers of 2009 and 2010, we conducted three experiments aiming to determine the role of vision in host selection by Warren root collar weevil. In 2009, host-seeking experiments were conducted in 16 square, outdoor bioassay plots measuring 1.5 × 1.5 m in an approximately 20 × 20 m area with bentgrass, *Agrostis* sp., vegetation in Prince George, British Columbia, Canada (53°51'43.2"N, 122°45'39.6"W). Each plot was surrounded by a 1-m tall polypropylene mesh wrapped around wooden corner stakes. The bottom 15 cm of the mesh was lined using duct tape with a slippery fluorocarbon polymer (2009: AD1070, AGC Chemicals Americas, Inc., Bayonne, New Jersey; 2010: Teflon PTFE, DuPont, Wilmington, Delaware) to prevent the weevils from climbing the mesh and escaping from the plot (Björklund, 2009). In addition, the bottom of the mesh was pinned to the ground with nails (length 5 cm) as a further measure to prevent weevil escape. The mesh and stakes were painted white to maximize the contrast of the silhouette treatment, inside the plot, against the background. Plots were located a minimum of 2 m apart.

Within each plot, two plastic flower pots (diameter 25 cm, depth 10 cm) were installed as pitfall traps. The traps were located 15 cm from the north and south sides of each plot such that the distance between the centres of the traps was 95 cm (Fig. 1A). Pitfall traps were placed so that their tops were flush with the ground. The top 4 cm inside the pitfall traps was coated with polymer to prevent captured weevils from climbing the sides and escaping.

In 2010, the study site was located at the Prince George Tree Improvement Station (53°46'18N, 122°43'4"W). This permitted enlargement of the plots to 2 × 4 m, spacing centres of the two pitfall traps in each arena 1 m from the fencing at both sides of the plot, and increasing the distance between centres of pitfall traps within a plot to 2 m (Fig. 1B). We judged this distance of 2 m to be more representative of the mean

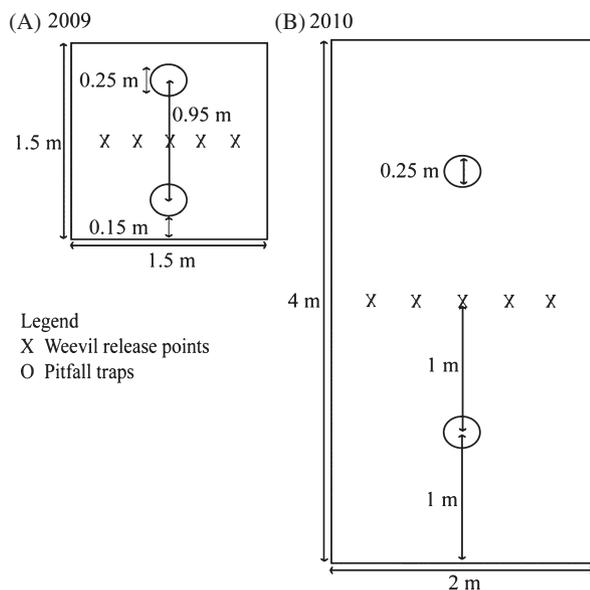


Figure 1 Schematic diagram of the design of outdoor bioassay plots used to investigate the role of vision in initial steps of adult Warren root collar weevils' host finding. Each plot had a treatment trap and an empty trap, except the control treatment, which had two empty traps. (A) Layout of 16 bioassay plots used in 2009 in Prince George, British Columbia, Canada (53°51'43.2N, 122°45'39.6"W). (B) Layout of 20 bioassay plots used in 2010 at the Prince George Tree Improvement Station, Prince George, British Columbia, Canada (53°46'18N, 122°43'4"W). Five weevils were released per plot.

distance that Warren root collar weevils can move in a night (Cerezke, 1994). We constructed 20 plots in 2010.

In both years, we tested weevil responses by erecting a dummy host-tree, which provided a visual stimulus in the form of a vertical silhouette in one of the two pitfall traps, chosen randomly by a coin toss, within each arena. Different types of silhouettes were used to emulate different potential hosts, and thus defined the experimental treatment. The specific treatments are described further below. The silhouette was erected in the middle of the pitfall trap such that it did not contact the ground outside of the trap, preventing the insects from climbing it without first entering the pitfall trap. The treatments were randomly assigned to the plots. Control treatments did not receive a silhouette (i.e. both pitfall traps were empty in those arenas).

Study organisms

Björklund funnel traps were made using tar paper (GAP Roofing Black #30 Roofing Felt, GAP Roofing, Inc., Pryor, Oklahoma) and installed on 167 lodgepole pine trees in a 10–20-year-old lodgepole pine stand in 2009, and on 602 trees in similar stands (7–25 years) in 2010 near Prince George, British Columbia, Canada (53°55'N, 122°49'W) in accordance with the protocol previously described by Björklund (2009). In 2009, there were two sampling periods, whereas, in 2010, there was only a single, longer, sampling period. The traps were checked each morning, and yielded 324 adult weevils on

11 May to 17 June and 13–28 July 2009, and 519 adult weevils on 26 April to 18 August, 2010.

Captured weevils were retained in groups of eight in square plastic containers (15 × 15 × 5 cm) with a piece of moist paper towel and a few small lodgepole pine branches for food (Toivonen & Viiri, 2006; Hopkins *et al.*, 2009). Food was changed twice per week, as required. The weevils were stored in an environmental growth chamber under a LD 12 : 12 h photoperiod at 8 °C and 75% relative humidity. The temperature was set at 8 °C to slow weevil metabolism (Toivonen & Viiri, 2006).

The sex of the weevils was determined using two non-invasive techniques described previously by Öhrn *et al.* (2008). Internal markings on the eighth sternite were also examined to increase confidence in the accuracy of the identification (G. R. Hopkins, M. D. Klingenberg and B. H. Aukema, unpublished data). Male and female weevils were then divided into separate plastic containers, again in groups of eight.

Experimental trials

In both years, all weevils utilized for an experiment had been captured that season. We marked each insect because the recapture rate of weevils released was not 100% and plots were reused for numerous trials. Marking was conducted by etching the elytra with a rotary drill as described by Klingenberg *et al.* (2010a), comprising a technique that has been successfully used on other ground-dwelling Coleoptera (Winder, 2004). Etchings were then filled in with nontoxic latex-based paint (Citadel Colour, U.K.) to accentuate markings (Klingenberg *et al.*, 2010a).

Before each experimental trial, weevils were placed in clean plastic containers without food for a period of 24 h. Containers were kept at ambient temperatures and exposed to the natural photoperiod in the range 12.8–14.2 h/day in 2009 to 14.3–17.0 h/day in 2010 depending on the season (National Research Council of Canada, 2011). For each experiment, five weevils were released along the centre line; once per plot per trial. This density reflects the highest weevil density commonly seen on single hosts in 20–25-year stands (Cerezke, 1994). Each trial ran 60 h. Pitfall traps were checked every 12 h for the duration of the trial. When a weevil was found in a pitfall trap, the replicate and treatment were recorded. The weevil was subsequently removed from the experiment. Insects were not reused.

Experiment 1: are weevils attracted to visual cues?

This experiment investigated the response of Warren root collar weevils to four silhouette treatments: crown, trunk, tree (crown + trunk) and control. The crown treatment consisted of a plastic Christmas tree (height 138 cm, trunk diameter 3 cm). The trunk treatment consisted of a piece of acrylonitrile butadiene styrene (ABS) pipe (height 90 cm, diameter 10 cm). The tree treatment consisted of a piece of ABS pipe (height 90 cm, diameter 10 cm), with a plastic Christmas tree (height 138 cm) inserted to give the appearance of the pipe being the trunk of the tree. The combined height of the ABS pipe

and Christmas tree was approximately 188 cm as a result of part of the Christmas tree being inserted into the ABS pipe. The control treatment had no silhouettes in either pitfall trap. In 2009, artificial Christmas trees were procured from garage sales in the Prince George metropolitan area. In 2010, the artificial trees were identical 'Canadian pine' specimens (original height 200 cm, crown height 104.1 cm), which were purchased from Wal-Mart (Canada). Despite the name, the trees had a visual appearance of a unique hybrid of pine and spruce; hosts acceptable to *H. warreni*. The trees had seven rows of six to eight branches. The branches decreased in length from the bottom of the tree to the top; the largest branches were 53 cm and the shortest were 27 cm. The branches had 10–16 'shoots', and the shoots were covered in 3-cm long plastic 'needles'. All plastic trees were unboxed and exposed to the air for over 48 h before use in experiments, although no associated odours were detectable to the human nose during the experiment set-up. Replicated trials were conducted from 25 to 28 August and 29 to 31 August 2009 and from 17 to 19 June and 23 to 25 June, 2010.

In 2009, a logistic mixed-effects analysis of variance was used to examine the effect of silhouette treatments on the number of weevils captured in control versus pitfall trap treatments (a binary response). Plot was the unit of replication. Fixed effects included terms for crown and trunk, as well as their interaction, whereas a term for plot was modelled as a random effect. Where significant differences between treatments existed (using $\alpha = 0.05$), means comparisons were performed using protected *t*-tests (Carmer & Swanson, 1973). In addition, a chi-square contingency analysis was conducted to determine whether there was a difference in the number of weevils caught in pitfall traps baited with the different treatment types. In 2010, with expanded plot size, no weevils were caught in empty traps within the treatment plots (see Results). A lack of weevils in these traps created an analytical challenge in estimating the proportion of weevils attracted to control versus treatments and the standard errors of those estimates in a binomial model framework because only treatments with a silhouette elicited positive responses. Hence, in 2010, we used chi-square contingency analysis to determine whether there was a difference in the number of weevils caught in pitfall traps with the different treatment types. All data analyses were performed in R, version 2.12.2 (R Development Core Team, 2011).

Experiment 2: does blinding remove host-finding ability?

This experiment investigated whether vision was a mechanism used by Warren root collar weevil in host orientation. Each plot was set up with two pitfall traps as described for the 2010 experiments above. One pitfall trap contained an artificial Christmas tree crown inserted into an ABS pipe to serve as a tree silhouette (tree treatment); the other was left empty (control). Five weevils were released in each of the 20 bioassay plots as described previously. In half the plots, blinded weevils were released; in the other half, nonblinded weevils were released.

Weevils were blinded using nontoxic Elmer's All Ceramic and Glass Cement mixed with lamp black nontoxic acrylic paint

(Americana, Elmer's, Columbus, Ohio). Blinding insects by applying paint to their eyes has confirmed use of vision in silhouette location by common field grasshoppers *Chorthippus brunneus* Thunberg (Kral, 2008), as well as prey-finding behaviour by a number of predaceous insects (Rossel, 1986; Awan *et al.*, 1989; Claver & Ambrose, 2001). The glue-paint mixture was applied to the weevils' eyes using a synthetic 000 S/H round paintbrush (Winsor & Newton University Series, U.K.). Trials were run from 12 to 14 September, 2009, 11 to 13 June, 2010 and 6 to 8 July, 2010.

In 2009, a likelihood ratio test (*G*-test) was used to examine the effect of blinding on the number of weevils found in empty pitfall traps versus pitfall traps underneath tree silhouettes. The test statistic of the *G*-test is distributed according to a chi-square distribution, and can be used when expected values are less than five in the contingency table (Gotelli & Ellison, 2004).

As in Experiment 1, the larger plots in 2010 resulted in very few insects being captured in the empty controls (see Results). Hence, we focused only on insects captured in pitfall traps with a tree silhouette. When inspecting weevils post-capture under a dissecting microscope, we found that some of the weevils' eyes were only partially covered with paint, either as a result of burrowing activity or scratching with tarsi. Because we could not conclusively confirm that all weevils were 100% blinded, we analyzed the likelihood of a weevil being captured in a trap with a tree silhouette as a function of vision impaired by paint versus nonblinded. In this logistic regression analysis, the statistical test associated with estimate of the intercept divided by its standard error yields a *Z*-statistic reflecting a test of whether the probability of recovering a nonblinded weevil in a silhouette baited trap was significantly different from 50% (i.e. reflecting random movement) [the test of whether the intercept was significantly different from zero, back-transformed by the logit link function, $\exp^0/(1 + \exp^0)$, reflects $H_0: P(\text{recovered specimen has paint on its eyes}) = 0.50$]. Again, a term for plot was included as a random effect. Data analysis was performed in R, version 2.12.2 (R Development Core Team, 2011).

Experiment 3: are weevils attracted to colour?

Experiments looking at the effects of white/black trunks and white/green crowns on Warren root collar weevil host selection were conducted during the summer of 2010. Four treatments were investigated. The treatments were 'trees' constructed with a white trunk and a green crown, a white trunk and a white crown, a black trunk and a white crown, and a black trunk and a green crown. The white trunks were composed of polyvinyl chloride (PVC) pipe (height 90 cm, diameter 10 cm), and black trunks were composed of similar ABS pipe. The white crowns were painted twice with white acrylic, latex, exterior, flat paint (BEHR Premium Plus Ultra Pure White Co., Santa Ana, California). The crowns of the green artificial Christmas trees were left unpainted. Again, each treatment was characterized by a silhouette placed into a pitfall trap opposing an empty pitfall trap in the other side of the enclosure plot. Two replicates of each of the four treatments were conducted at a time. Four trials were conducted: 3–5 August, 9–11 August, 12–14 August and 21–23 August, 2010.

Before carrying out the present study, we conducted a preliminary study investigating the effects of paint on weevil behaviour. In that experiment, weevils were given a choice between two similar tree silhouettes: one crown was painted with a matching green colour of the original plastic branches, whereas the other was left unpainted (= green). A logistic regression model was used to determine whether paint affected the number of weevils caught in the pitfall traps surrounding both trees ($Z = -1.24$, $P = 0.215$). Thus, we concluded that paint, at least in the tree crowns, did not affect weevil behaviour (L. Machial, unpublished data).

As a result of the tendency of weevils to avoid empty pitfall traps in the larger plots in 2010, we again focused solely on the number of weevils caught in pitfall traps surrounding the four, coloured silhouettes. A chi-square contingency analysis was conducted to determine whether there was a difference among treatments. Data analysis was performed in R, version 2.12.2 (R Development Core Team, 2011).

Results

Experiment 1: are weevils attracted to visual cues?

Weevils demonstrated clear attraction to vertical silhouettes in both 2009 and 2010, although the type of silhouette eliciting attraction was not necessarily consistent between years (Fig. 2). For example, in 2009, the proportion of weevils falling into the two pitfall traps in the control plots was not significantly different from 50% (i.e. a random pattern of dispersion in the absence of vertical silhouettes). The crown and trunk treatments did not differ from controls, although the tree treatment that combined the trunk and crown silhouette captured significantly more weevils than the associated empty trap ($F_{1,30} = 5.48$; $P = 0.0259$; Fig. 2A). In addition, when the proportion of weevils found in pitfall traps baited with silhouettes was directly compared, more weevils were found in pitfall traps baited with tree silhouettes than in crown, trunk or the empty control pitfall trap ($\chi^2 = 10$, d.f. = 3, $P = 0.0186$).

In 2010, proportionately more weevils were found in the pitfall traps surrounding crown, trunk and tree silhouettes than in the empty control pitfall traps ($\chi^2 = 13.7$, d.f. = 3, $P = 0.004$; Fig. 2B). Indeed, not a single weevil was captured in the empty trap in any of the plots containing a silhouette. There was no preference for tree silhouettes over crown or trunk silhouettes ($\chi^2 = 0.316$, d.f. = 2, $P = 0.85$). Sex did not affect host-orientation behaviour because there was no difference in the response of male and female weevils to various silhouettes for either year (2009: $F_{1,29} = 0.22$; $P = 0.64$; 2010: $\chi^2 = 4.83$, d.f. = 3, $P = 0.69$).

Experiment 2: does blinding remove host-finding ability of weevils?

Application of paint to eyes of the insects altered their attraction to vertical silhouettes. In a preliminary experiment conducted in 2009, 17 of the 40 weevils originally released into the plots were recaptured in the pitfall traps. The proportions found in the

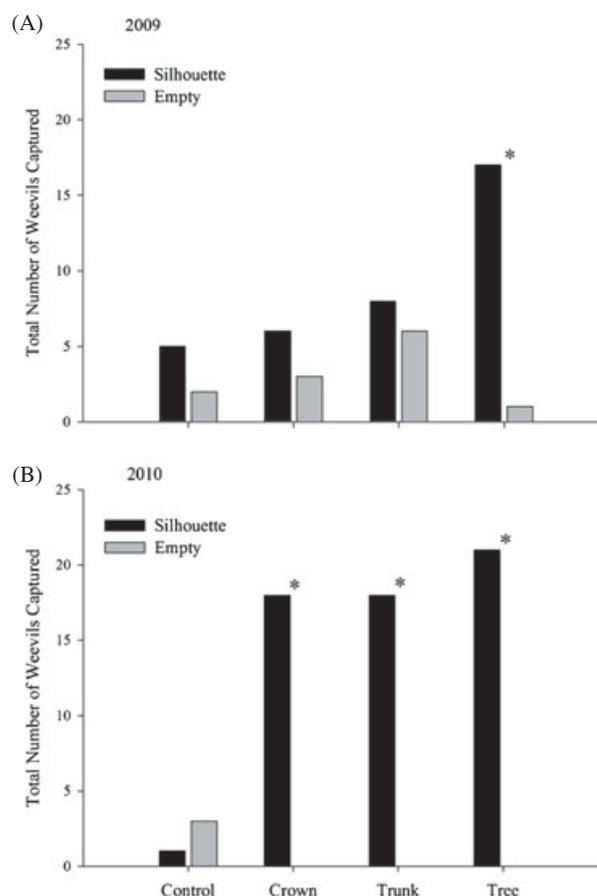


Figure 2 Total number of weevils of Warren root collar weevils captured in pitfall traps in each of four silhouette treatments; tree, crown, trunk and control. Each plot had a treatment trap and an empty trap, except the control treatment, which had two empty traps. (A) Experiments were conducted from 25 to 31 August 2009 ($n = 40$ weevils per treatment) (five insects \times four replicates \times two trials). An asterisk indicates a statistically significant difference in proportions ($F_{1,30} = 5.48$; $P = 0.0259$). (B) Experiments were conducted from 17 to 25 June 2010 ($n = 50$ weevils per treatment) (five insects \times five replicates \times two trials). An asterisk indicates a statistically significant number of insects trapped in silhouette treatments versus control ($\chi^2 = 13.7$, d.f. = 3, $P = 0.004$).

treatment versus empty controls differed significantly among blinded and nonblinded insects ($G_{\text{adjusted}} = 5.35$, d.f. = 1, $P = 0.0207$; Fig. 3). Of the 17 weevils recaptured, seven were blinded and 10 were not. The seven blinded weevils were found in both empty pitfall traps ($n = 3$) and those baited with a tree silhouette ($n = 4$). By contrast, all 10 of the nonblinded weevils were found in pitfall traps with silhouettes. This pattern of host-location persisted in the more robust experiment conducted in 2010.

In 2010, only three weevils of the 100 released over the course of the assays were found in the empty control. Fifty-three of them were found in the traps beneath tree silhouettes. Insects found in these traps were more frequently nonblinded (84.9%) versus blinded (15.1%) ($Z = -4.52$, $P < 0.0001$; Fig. 4).

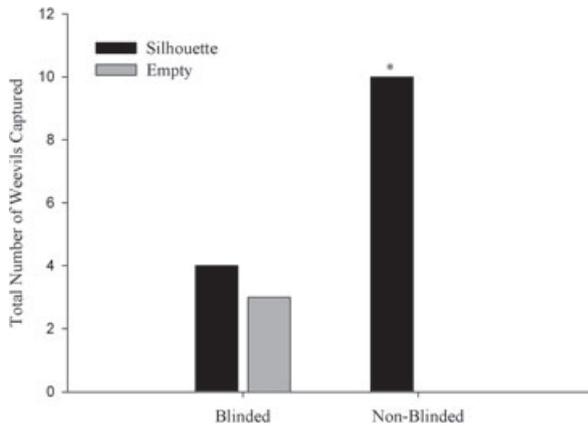


Figure 3 Total number of blinded and nonblinded Warren root collar weevils captured in empty pitfall traps and pitfall traps with tree silhouettes. Each plot had a treatment trap and an empty trap, except the control treatment, which had two empty traps. Experiments were conducted from 12 to 14 September 2009 ($n = 20$ weevils per treatment) (five insects \times four replicates \times two trials). An asterisk indicates a statistically significant difference in proportions ($G_{\text{adjusted}} = 5.35$, d.f. = 1, $P = 0.0207$).

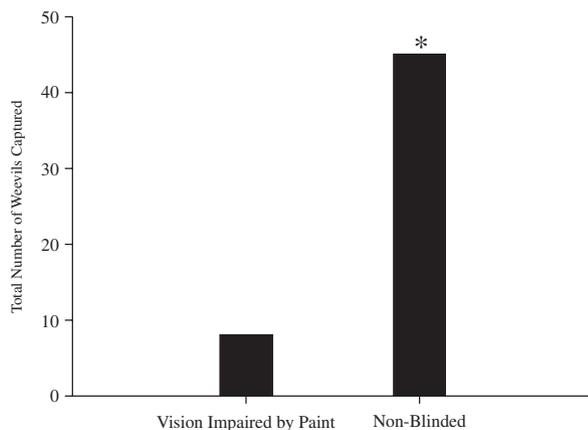


Figure 4 Total number of Warren root collar weevils with vision impaired by paint versus nonblinded controls captured in pitfall traps with tree silhouettes. Experiments were conducted from 11 to 13 June and 6 to 8 July 2010 ($n = 50$ weevils per treatment) (five insects \times five replicates \times two trials). An asterisk indicates a statistical significance difference from the other treatment ($Z = -4.52$, $P < 0.0001$).

Experiment 3: are weevils attracted to colour?

The attraction of weevils to silhouettes did not change when colours of the trunk and crown portions were manipulated. Only two out of 54 captured weevils were found in the empty control (Fig. 5). The insects demonstrated equal attraction to silhouettes composed of combinations of white/green crowns and white/black trunks ($\chi^2 = 0.4615$, d.f. = 3, $P = 0.93$).

Discussion

The finding that vision plays an important role in host finding behaviour by Warren root collar weevil is consistent with

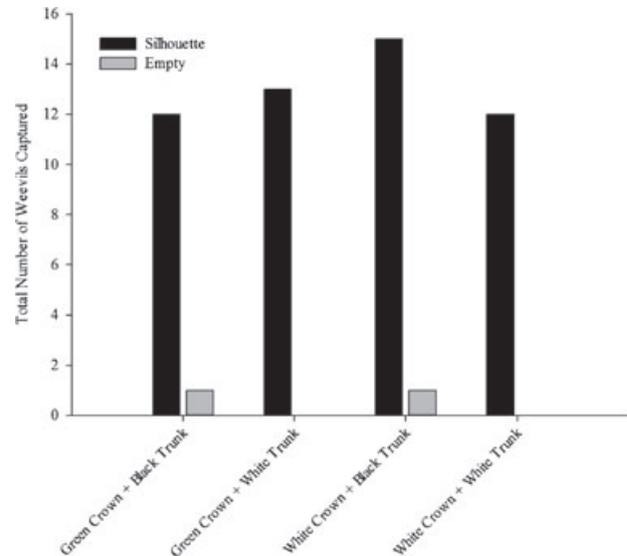


Figure 5 Total number of Warren root collar weevils captured in arena choice bioassays with a control pitfall trap and a pitfall trap baited with one of four silhouette treatments: green crown/black trunk, green crown/white trunk, white crown/black trunk and white crown/white trunk no significant difference among silhouette treatments; ($\chi^2 = 0.4615$, d.f. = 3, $P = 0.93$). Experiments were conducted from 3 to 23 August 2010 ($n = 40$ weevils per treatment) (five insects \times two replicates \times four trials).

behaviour observed in several other wood-boring insects that integrate visual cues of vertical silhouettes with other cues (Hunt & Raffa, 1991; Strom *et al.*, 1999; De Groot & Nott, 2001; Strom *et al.*, 2001; Goyer *et al.*, 2004; Campbell & Borden, 2006a, b; Campbell & Borden, 2009; Reeves, 2011) The strong role of vision in host orientation by Warren root collar weevil in the absence of any discernible chemical stimuli to date, however, is rare among root-boring insects. Among species of *Hylobius*, attraction to odourless visual stimuli has only been previously noted in large pine weevil *Hylobius abietis* L. (Björklund *et al.*, 2005). In most insects, responses to olfactory cues play a dominant role in host selection, and responses to visual cues only occur when appropriate chemical cues are present (Bernays & Chapman, 1994; Bernays, 2003). When searching for nondamaged conifer hosts, *H. abietis* is equally attracted to traps baited solely with chemicals or solely with visual stimuli; however, the strongest attraction is to traps baited with both chemical and visual stimuli (Björklund *et al.*, 2005). The distinctive response of Warren root collar weevil adults to visual stimuli in the absence of chemical stimuli may be explained, in part, by this insect's range of host plants and limited dispersal capabilities associated with its life-history strategy and mode of movement.

Warren root collar weevils are oligophagous, feeding on a variety of hosts in the Pinaceae family, including species of *Pinus*, *Picea*, *Abies*, *Larix* and *Tsuga* (Warren, 1956; Wood, 1957; Whitney, 1961; Wood & Van Sickle, 1989; Cerezke, 1994; Hopkins *et al.*, 2009). Oligophagous and monophagous insects tend to be visual specialists compared with polyphagous insects because plants within the same family are more likely to have similar morphologies than plants found in different

families (Prokopy & Owens, 1978). Plants showing similar morphologies allow the insects that feed on them to develop specific search images that aid in host location (Prokopy & Owens, 1978, 1983; Aluja & Prokopy, 1993; Stenberg & Ericson, 2007).

Visual cues are often important in short range host selection, from distances of a few centimetres up to 10 m (VanderSar & Borden, 1977; Bernays, 2003). Movement by adult Warren root collar weevils falls within this range because the insects traverse a mean distance of up to 2 m per night (Cerezke, 1994; Klingenberg *et al.*, 2010a; Machial *et al.*, 2012). Flightless insects may not be as dependent upon long-distance (i.e. chemical) cues if the insects typically exist within host pools not ephemeral in space and time. For example, bark beetles require olfactory capabilities to process a cacophony of competing host- and nonhost volatiles in flight as they seek either new live trees (in 'aggressive' tree-killing species) or stressed and weakened hosts (in 'secondary' species) (Huber *et al.*, 2000; Raffa, 2001). By contrast, Warren root collar weevil can persist for more than one generation on a mature coniferous tree without killing the host (Cerezke, 1994), and many suitable hosts of various species are often found together in a coniferous forest. After using visual stimuli to locate and arrive at a potential host, other sensory cues, such as tactile and gustatory stimuli, are likely to be incorporated into the host selection process (Bernays & Chapman, 1994). Exploitation of chemical cues for host location is likely critical for larval life stages, similar to *H. abietis* (Nordenhem & Nordlander, 1994; Nordlander *et al.*, 1997) because female Warren root collar weevil tend to be egg scatterers that deposit their eggs near potential hosts.

Our experiments were not designed to characterize seasonal variations in responses of adult Warren root collar weevils to host cues. Seasonal variations can result from changes in weevil age and reproductive status, causing shifts in the insects' biological requirements (Nordenhem & Eidmann, 1991; Hoffman *et al.*, 1997). For example, early in the season, the insects may be searching for hosts on which to feed before mating, whereas, late in the season, females may be looking for hosts near which to oviposit. Declining discrimination as female behaviour shifts from feeding to egg scattering (Minkenberg *et al.*, 1992; Cerezke, 1994; Hopkins *et al.*, 2009) is consistent with our results from 2009 to 2010 (Fig. 2), in which a higher level of discrimination among silhouettes in 2010 coincided with an earlier season assay (June 2010 versus August 2009). We were cautious when making comparisons between years, however, because the plots were enlarged in 2010. As such, the pattern in 2010 could simply have reflected shelter-seeking rather than a feeding or ovipositional strategy. In preliminary assays, however, we found that the insects would readily climb both PVC pipes and artificial Christmas trees, comprising behaviour more indicative of searching for food than avoiding predators or inclement weather.

Many insects that feed on conifer trees are more attracted to black silhouettes than to white ones (Dubbel *et al.*, 1985; Strom *et al.*, 1999; Campbell & Borden, 2006a, b; Campbell & Borden, 2009), putatively because black silhouettes more closely resemble the trunks of host trees, whereas white silhouettes more closely resemble the trunks of nonhost angiosperms. Throughout all experiments conducted in the

present study, weevils demonstrated a preference for full tree silhouettes, regardless of black, white or green colour combinations. The attraction to silhouettes may have masked weaker responses to colour because we did not directly compare colours in a choice assay. Alternatively, we may have failed to test the most optimal colour to which Warren root collar weevils are attracted. Because the insects are putatively nocturnal, future research should focus on colour contrast, spectral sensitivity and silhouette angle.

Currently, there is no easy and accurate method for estimating weevil population sizes in forests or plantations. Tree mortality may lag the appearance of high numbers of adult Warren root collar weevils by 2 or 3 years because larvae, the most damaging life stage to young trees, typically take 2 years to develop to adults (Cerezke, 1994). Furthermore, infestation levels are typically higher than what is apparent by mortality rates (Schroff *et al.*, 2006). As a result, high levels of damage may occur before it becomes apparent that populations of Warren root collar weevil are at critical levels. Our results suggest that a trap could potentially be developed by placing nondestructive tree-trunk funnel traps that capture live Warren root collar weevils known as Björklund traps around the base of plastic vertical silhouettes (Björklund, 2009). Using such apparatuses versus live trees could allow easy placement and transport within forested areas where they would be most effective, such as within harvested sites to reduce weevil numbers before replanting, or at the margins of young plantations to reduce weevil ingress (Klingenberg *et al.*, 2010b).

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