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Influence of trap colour, type, deployment height, and a host volatile on monitoring *Orchestes fagi* (Coleoptera: Curculionidae) in Nova Scotia, Canada[†]

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Abstract

Orchestes fagi (Linnaeus) (Coleoptera: Curculionidae) is a pest of beech trees (Fagus sylvatica Linnaeus; Fagaceae) in Europe that has recently become established and invasive on American beech (Fagus grandifolia Ehrhart) in Nova Scotia, Canada. We tested the effects of trap type, trap colour, trap height, and lure on the numbers of O. fagi captured per trap with the objective of developing a survey tool to monitor the weevil's spread. We captured O. fagi in significantly greater numbers on yellow, green, or white traps than on light blue, dark blue, or red traps. There were no significant interactions between trap colour and trap design. Sticky triangular prism traps caught significantly more O. fagi than did nonsticky intercept traps regardless of colour. No effect of trap height was observed. Mean catch of O. fagi was significantly greater on yellow sticky triangular prism traps than on commercially sourced yellow sticky cards. Baiting yellow, green, or white sticky prism traps with the host volatile 9-geranyl-p-cymene did not increase catch of O. fagi adults and monitoring the spread of this species in Canada.

Introduction

The beech leaf mining weevil, Orchestes fagi (Linnaeus) (Coleoptera: Curculionidae), is a common pest of European beech, Fagus sylvatica Linnaeus (Fagaceae), trees in Europe (Mangels et al. 2015). Adult O. fagi feed on developing leaves at the time of budburst, causing shot holes as the leaves mature; larvae mine the leaves from the mid-rib outwards, forming blotch mines at the leaf margin. Infested leaves are often stunted with necrotic tips that appear scorched (Bale 1984; Sweeney et al. 2012). The majority of leaf damage occurs in spring during leaf development (Bale 1984). Although O. fagi is considered the most abundant herbivore on F. sylvatica in Europe (Bignucolo and Korner 2010) and occasional outbreaks may reduce tree growth and beechnut production, tree mortality is not reported (Verkaik et al. 2009).

Orchestes fagi was discovered infesting American beech, Fagus grandifolia Ehrhart (Fagaceae), in Nova Scotia, Canada, in 2012, but was likely established at least five years prior to its discovery based on anecdotal reports of beech defoliation (Sweeney *et al.* 2012). Infested American beech trees often respond to leaf damage by producing a second flush of leaves (J.T.L.G., personal observation).

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American beech in the Halifax, Nova Scotia area have sustained heavy *O. fagi* damage for six or more consecutive years and many mature trees have died in both natural stands and residential areas (Sweeney and Johns 2016; J.T.L.G., personal observation).

The risk that *O. fagi* may be spread long distances by human movement of logs and firewood is high because adult *O. fagi* overwinter in large numbers on the trunks of trees, including beech, spruce (*Picea* Dietrich; Pinaceae), and maple (*Acer* Linnaeus; Sapindaceae) (Morrison *et al.* 2017).

There are already several populations of *O. fagi* now established in areas of Nova Scotia hundreds of kilometres from Halifax, such as Cape Breton (Sweeney *et al.* 2012). The relatively rapid rate of spread of *O. fagi* combined with significant tree mortality in infested stands indicates that *O. fagi* is a substantial threat to American beech, which is already in decline throughout its range in eastern North America due to beech bark disease, *Nectria coccinea* var. *faginata* Lohman, Watson, and Ayers (Nectriaceae) (Houston and O'Brien 1983; Sweeney *et al.* 2012). We examined trap colour, trap height, and trap type preferences of *O. fagi* with the goal of developing an effective method of monitoring the spread of *O. fagi* in North America.

Historically, when developing trapping methods for phytophagous insects, visual cues have often been considered less important than olfactory cues for insect attraction (Reeves 2011), likely due to species specificity of olfactory cues. However, vision often plays a large role in host location by phytophagous insects (Brattli *et al.* 1998). Preliminary laboratory bioassays employing a colour choice tube found that *O. fagi* was attracted to the blue, green, red, and white colours (Pawlowski 2017). To further explore trap colour preference in *O. fagi*, we compared six different colours of traps to determine which colours were most attractive.

Insects are often more sensitive to the shape of a visual stimulus than its colour (Reeves and Lorch 2009; Machial *et al.* 2012). For example, in the Warren root collar weevil, *Hylobius warreni* Wood (Coleoptera: Curculionidae), host attraction was affected by the shape of the stimulus but not its colour (Machial *et al.* 2012). Previous trapping trials for *O. fagi* employed yellow sticky card traps baited with semiochemicals and deployed in the low canopy of beech trees (Pawlowski 2014). In this study, we compare three trap types to assess the efficacy of each trap type on attraction, as measured by trap catch: (1) nonsticky panel traps, (2) mini-prism sticky traps, and (3) traditional yellow sticky card traps would outperform mini-prism traps in mean trap capture due to greater trap surface area.

Orchestes fagi females tend to oviposit in the lower canopy and feed in the upper canopy (Phillipson and Thompson 1983). Therefore, we predicted that traps in the upper canopy would catch more *O. fagi* than traps in the lower canopy.

Grimm (1990) showed that *O. fagi* were attracted to bursting beech buds. This behaviour is adaptive as survival of first instars decreases as beech leaves mature and sclerotise (Bale 1984). Silk *et al.* (2017) found that 9-geranyl-*p*-cymene, a compound emitted in increased quantities from beech leaves at the time of budburst, increased catch on sticky card traps compared to unbaited controls and caused upwind movement in olfactometer studies. In the present study, we predicted that mini-prism traps baited with 9-geranyl-*p*-cymene lures would catch more *O. fagi* than unbaited controls.

Methods

Trap design

Mini triangular prism traps (hereafter referred to as prism traps) were constructed using 20-cmwide and 40-cm-long sections cut from 122 cm \times 244 cm sheets of corrugated polypropylene plastic. Except for light green (ultraviolet green-Pantone 360C, Laird Plastics, Moncton, New Brunswick, Canada), all colours of corrugated plastic (red-Pantone 186C, yellow-Pantone 123C, dark blue-Pantone 289C, light blue-Pantone 285C, and white-Pantone NA) were sourced from Sabic

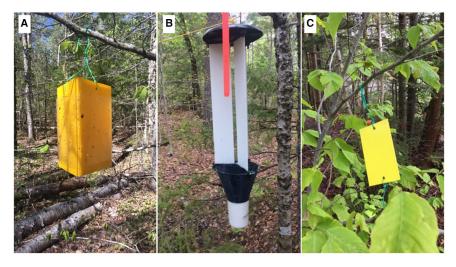


Fig. 1. Different traps used. **A**, Yellow triangular prism trap, June 2017, Oakfield Provincial Park, Oakfield, Nova Scotia; **B**, white panel trap, June 2017, Oakfield Provincial Park, Oakfield, Nova Scotia; **C**, yellow sticky card, June 2018, Wolfville, Nova Scotia. Photographs by J. Goodwin.

Polymershapes (Dartmouth, Nova Scotia, Canada), a distributer of Coroplast (Granby, Québec, Canada). Strips were bent to form hollow triangular prisms with sides measuring 12.5 cm wide and 20 cm tall (Fig. 1A). Prisms were wrapped with Alpha Scents adhesive roll tape (Alpha Scents Incorporated, Portland, Oregon, United States of America) to immobilise insects. The advantages of the adhesive roll tape over tangle-trap are: (1) it made for efficient trap maintenance and reuse of the corrugated plastic prism traps (by simply replacing a bug-covered sticky strip with a fresh one, as described below); and (2) unlike tangle-trap, the sticky material on the adhesive roll does not get stuck on one's hands and fingers.

Synergy Semiochemicals Multitrap Panel traps (Synergy Semiochemicals Corporation, Delta, British Columbia, Canada) (hereafter referred to as panel traps) had three corrugated plastic panels (each 11 cm \times 61 cm) overtop of a funnel (22.5 cm top diameter 11.5 cm bottom diameter) and collecting cup (11.5 cm diameter). These traps allowed us to choose any available colour of corrugated plastic for the panels but we used a black trap top and funnel and a white collecting cup for all panel traps. The panels, and inside and outside surfaces of the funnels were coated in Fluon to reduce friction and increase catch (Graham *et al.* 2012; Allison and Redak 2017). Collecting cups contained a saturated saltwater solution (Fig. 1B).

Yellow sticky cards (number 611; bright yellow; Contech Incorporated, Delta, British Columbia, Canada) (hereafter referred to as card traps) were 13 cm \times 7 cm and were pretreated with Tanglefoot Tangle-Trap sticky coating on both sides (The Scotts Company, Marysville, Ohio, United States of America) (Fig. 1C). Holes were punched in the top and bottom centre of each card to allow for hanging.

Trap deployment and replacement

Unless stated otherwise, traps were deployed at shoulder height (approximately 1.5 m) from branches of American beech in a randomised block design with at least 5 m between traps and blocks. Traps were checked weekly in 2017 and biweekly in 2018. Prism traps and card traps were replaced when the sticky surface was compromised with bycatch or if the trap had caught at least one *O. fagi*, as follows: a new trap was put in place of the used trap and the wax paper strip was removed to expose the sticky surface; the wax paper strip was then used to cover the sticky surface of the used traps were transported back to the laboratory. Used traps were transported back to the



Fig. 2. Various colours of triangular prism trap used in the study. Left to right: light blue, red, green, yellow, white, and dark blue. Acadia University, Wolfville, Nova Scotia. Photograph by J. Goodwin.

laboratory. Captured *O. fagi* were removed from the sticky traps in the laboratory using Histo-Clear (HistoClear II; National Diagnostics, Atlanta, Georgia, United States of America). Any *O. fagi* caught in panel traps were removed using a strainer, and the saltwater in each trap cup was replenished weekly. From 2017 trap catch, the total number of males and females was recorded (beetles were sexed according to Pawlowski 2017), while in 2018 a subsample of six *O. fagi* from each trap catch was sexed. Beetles were identified according to Sweeney *et al.* (2012). Voucher specimens have been deposited in the insect collection at the Atlantic Forestry Centre, Fredericton, New Brunswick, Canada.

Trap colour preference experiment

Trap colour preference was explored using prism traps of six different colours (yellow, green, white, dark blue, light blue, and red) (for colour spectra see Fig. 7) (Fig. 2) with eight replicates per treatment in Oakfield Provincial Park, Oakfield, Nova Scotia, Canada (44.9174°N, 63.5862°W), from 1 May through 28 July 2017.

Prism trap versus panel trap experiment

Trap type preference was explored in a 2×3 factorial experiment with two types of traps (panel traps and prism traps) of three different colours (green, white, and light blue), and eight replicates per treatment in Oakfield Provincial Park, from 18 May through 28 July 2017.

Trap height preference experiment

Trap height preference was explored in a 2 × 2 factorial experiment with two colours of prism traps (green and light blue) placed either low (1.5 m) or high (\geq 3 m) in the canopy of trees in a beech stand at the K.C. Irving Centre gardens and Acadia University Woodland Trails in Wolfville, Nova Scotia (45.087489°N, 64.368563°W) from 31 May through 27 July 2017. We chose green and light blue traps for this experiment based on Pawlowski (2017) who found green and blue attractive to *O. fagi*. For each colour, a low and high trap were placed in each of five trees, that is, five trees with green traps and five trees with blue traps.

Trap colour and host volatile interaction experiment

In 2018, we tested the three most attractive colours (as determined in 2017: yellow, green, and white) with and without 9-geranyl-*p*-cymene in a 3×2 factorial experiment replicated eight times

in Oakfield Provincial Park from 7 May to 2 August 2018. 9-geranyl-*p*-cymene was synthesised at the Canadian Forest service using the protocol developed by Silk *et al.* (2017) and loaded on rubber septa (Septa – Raw Red; Scotts Canada, Delta, British Columbia, Canada) at 2 mg per lure, with a release rate of approximately 20 μ g/day at 20 °C.

Prism trap versus sticky card experiment

In this experiment, we tested the most attractive colour (yellow) and most effective trap type (prism) from 2017 against commercially available yellow sticky cards to determine their efficacy at detecting *O. fagi* across a range of population densities. Twenty pairs of traps were deployed from 10 May to 2 August 2018. All traps were baited with 2 mg of 9-geranyl-*p*-cymene on rubber septa. Pairs of traps were deployed in three locations: Uniacke Estate Museum Park, Mount Uniacke, Nova Scotia (10 pairs) (44.896358°N, 63.834614°W); a residential property in New Ross, Nova Scotia (five pairs) (44.736965°N, 64.456596°W); and at the K.C. Irving Centre gardens and Acadia University Woodland Trails (five pairs). We did not measure the reflectance spectrum from the yellow sticky cards but based on published spectra they likely had a sigmoidal reflectance curve asymptotic at about 570 nm typical for bright yellow (Patt and Sétamou 2010; Silva *et al.* 2018).

Statistical analyses

All statistical analyses were completed using R (RStudio Team 2016), with total catch per trap over the entire trapping period as the response variable. If a trap was downed during a week of the study, we removed data from all traps from that block and week from analysis. Relationships among variables were assessed with generalised linear mixed models (GLMM; lme4 package) using the appropriate distribution. Negative binomial generalised linear mixed models were used to assess data from the trap colour preference and prism trap versus sticky card experiments, Gaussian generalised linear mixed models were used to assess data from the trap colour and host volatile interaction experiment, and the log(catch + 1)-transformed data from prism trap versus intercept trap experiment. Model fit was assessed using residual plots, Akaike information criterion values, and Hosmer–Lemeshow goodness-of-fit tests (ResourceSelection package) (Archer *et al.* 2007). Tukey's *post hoc* tests (emmeans package) were used to determine whether differences in mean catch were significant among levels of each fixed effect (*i.e.*, among different colours or trap types) (Ward *et al.* 2019).

To analyse data from the trap height preference experiment, we used a generalised linear model and the distribution with best fit (negative binomial) to assess differences in mean catch between trap height (colours pooled) and the Wilcoxon rank-sum test to assess differences in mean catch between colours. We used the Wilcoxon rank-sum test to assess differences in total catch for each colour. Type 1 error rate was controlled at 5% for all analyses ($\alpha = 0.05$). A χ^2 goodness-of-fit test was conducted to test for sex bias in overall trap catch.

Results

There was a significant sex bias in trap catch across all experiments, χ^2 (1, n = 10886, P < 0.001), with females representing approximately 60–65% of *O. fagi* caught overall.

Trap colour preference experiment

We caught a total of 4893 *O. fagi* over the 13-week trapping period. Colour had an effect on trap catch (F = 18.2; df = 5, 35; P < 0.001). Total trap catch in each treatment was significantly

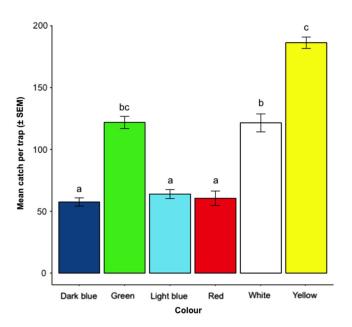


Fig. 3. Mean \pm standard error of the mean (SEM) number of *Orchestes fagi* adults caught per prism trap of each colour throughout the entire trapping season (1 May–28 July 2017) (n = 4893). Different letters denote significant differences between means (Tukey's test, P < 0.05).

greater on yellow traps, followed by white and green, with lowest catches on blue and red traps (Fig. 3).

Prism trap versus intercept trap experiment

We caught 3206 *O. fagi* over the 10-week trapping period. Prism traps caught significantly more *O. fagi* than panel traps (F = 17.2; df = 1, 35; P < 0.001), but neither trap colour (F = 1.15; df = 2, 35; P = 0.328) nor the interaction between trap colour and trap type (F = 2.35; df = 2, 35; P = 0.110) was significant (Fig. 4).

Trap height preference experiment

We caught 1265 *O. fagi* over the nine-week trapping period (31 May–27 July 2017). Mean catch was greater in high traps than low traps (trap colours pooled) (F = 4.21; df = 1, 18; P = 0.055) but did not differ significantly between green and blue traps (heights pooled) (W(4) = 9, P = 0.548) (Fig. 5).

Trap colour and host volatile interaction experiment

We caught 3566 *O. fagi* through the course of this 12-week trapping experiment. Trap catch did not differ significantly with colour (F = 1.06; df = 2, 35; P = 0.356), lure (F = 0.428; df = 1, 35; P = 0.517), nor interaction between the two (F = 0.281; df = 2, 35; P = 0.757) (Fig. 6).

Prism trap versus sticky card experiment

We caught 1377 *O. fagi* during this 12-week experiment. Mean catch (\pm standard error) on prism traps (56.1 \pm 7.3) was significantly greater than mean catch on sticky cards (12.8 \pm 2.3) (*F* = 92.6; df = 1, 38; *P* < 0.0001). However, after controlling for area of sticky surface (*i.e.*, mean catch per square centimetre), there was no difference in trap catch between trap types (*F* = 0.15; df = 1, 38; *P* = 0.70).

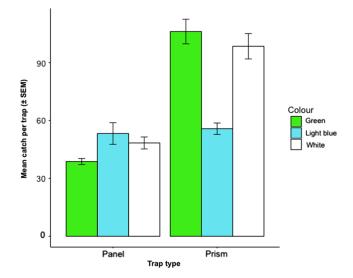


Fig. 4. Mean ± standard error of the mean (SEM) number of *Orchestes fagi* adults captured per trap of each colour and type throughout the entire trapping season (18 May–28 July 2017) (n = 3206). Prism traps caught more *O. fagi* than panel traps (F = 17.2; df = 1, 35; P < 0.001). No effect of colour or interaction between colour and trap type was observed.

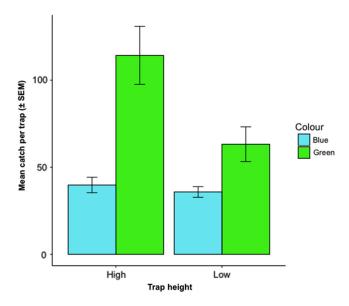


Fig. 5. Mean \pm standard error of the mean (SEM) number of *Orchestes fagi* adults captured per prism trap of each colour and height throughout the entire trapping season (31 May–27 July 2017) (n = 1265). Mean catch was greater in high traps than low traps (heights pooled) (F = 4.21; df = 1, 18; P = 0.055) but did not differ between green and blue traps (heights pooled) (W(4) = 9, P = 0.548).

Discussion

We found that yellow, green, or white traps captured more *O. fagi* adults than did red and blue traps. Our results differ somewhat from those from laboratory bioassays by Pawlowski (2017) in which *O. fagi* was attracted to blue as well as green and white. However, it is difficult to directly compare our results with those of Pawlowski (2017) due to differing conditions between laboratory and field bioassays. The laboratory bioassays used Creatology colour foam

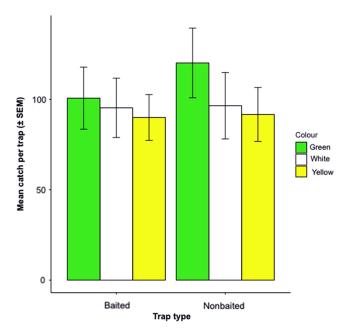


Fig. 6. Mean \pm standard error of the mean (SEM) number of *Orchestes fagi* adults captured per green, yellow, or white sticky prism trap of each colour that were baited with a 2-mg 9-geranyl-*p*-cymene lure or unbaited (7 May–2 August 2018) (n = 3566). No significant differences were found between treatment groups.

strips (Michaels, Irving, Texas, United States of America) placed directly adjacent to one another inside a 40-cm diameter cylinder with an overhead light-emitting diode full spectrum light source (Pawlowski 2017), whereas our corrugated plastic traps were spaced at least 5 m apart in natural sunlight. In terms of potential efficacy as survey tools, we have more confidence in results from field bioassays.

The colour yellow is considered to be universally attractive to all foliage-seeking insects (Prokopy and Owens 1983). The wavelength of yellow ranges from 570-585 nm, while green ranges from 490-570 nm (Reusch 2013). The corrugated plastic we used had peak wavelengths of 540 and 590 nm for green and yellow, respectively (Fig. 7). Orchestes fagi may be attracted to colours in the 540–590 nm range (green-yellow) since they resemble the colour of healthy foliage. The peak reflectance frequency of white corrugated plastic was 440 nm, in the violet range of the visual spectrum (Reusch 2013), but also had high reflectance values in the green-yellow range of the spectrum (Fig. 7). Hausmann et al. (2004) suggested a trichromatic visual system in Anthonomus pomorum (Linnaeus) (Coleoptera: Curculionidae), with sensitivities to ultraviolet (350 nm), blue (455 nm), and green (550 nm) light. The most preferred colours by O. fagi, yellow, green, and white, closely relate to the area of the visible spectrum, which was attractive to A. pomorum (Reusch 2013), perhaps because both species are phytophagous and rely on similar host location mechanisms. The emerald ash borer, Agrilus planipennis Fairmaire (Coleoptera: Buprestidae), is sensitive to light in the ultraviolet (420–430 nm), violet (460 nm), and green (530–560 nm) range of the electromagnetic spectrum (Crook et al. 2009). Tests of physiological sensitivity would need to be conducted to determine the retinal response of O. fagi to different wavelengths of light (i.e., electroretinography). Future studies should test the attraction of O. fagi to traps with peak reflectance wavelengths in the violet portion of the spectrum.

Our prediction that mean trap catch would be directly related to trap surface area was not supported. Prism traps significantly outperformed panel traps and card traps despite having a surface area that was intermediate between the other trap types. The success of this trap type over others

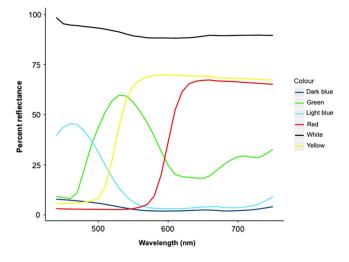


Fig. 7. Reflectance values from spectral analysis of coloured corrugated plastic used in trapping experiments. Measured using HunterLab UltraScan XE Reflected Color Spectrophotometer at Acadia University (Artisan Technology Group, Champaign, Illinois, Unites States of America).

could be attributed to its shape. Trap type or shape can affect the types of insects and numbers caught (Chénier and Philogène 1989; Sweeney *et al.* 2006; Dodds *et al.* 2010; Graham *et al.* 2012). However, catch was positively linked to surface area in our prism trap versus card trap experiment. Prism traps have approximately three times the surface area of card traps and when mean catch per surface area was compared between these two trap types, much of the difference in catch was accounted for by surface area. Therefore, the difference in trap catch between prism traps and panel traps must be due to a factor other than surface area. One explanation for the decreased effectiveness of panel traps compared with prism traps is the mechanism of action of the trap. When comparing sticky stovepipe traps with intercept traps, Chénier and Philogène (1989) describe larger trap catch of forest Coleoptera using sticky traps. A sticky trap surface may retain a greater proportion of attracted beetles than does a panel trap. However, we replaced traps every one or two weeks, reducing the chances of saturating trap surface area. Our results may have been affected if traps were replaced less frequently, for example, under seasonal deployment conditions.

We predicted that traps placed higher in the canopy would capture more *O. fagi* than those placed low in the canopy due to a preference of adult *O. fagi* to feed in the upper canopy (Phillipson and Thompson 1983) and our results supported our prediction, though significance was marginal (P = 0.055).

Our finding that baiting traps with 9-geranyl-*p*-cymene lures did not affect mean trap catch conflicts with results determined by Silk *et al.* (2017) even though lures were loaded with the same initial dosage of compound in both studies. Discrepancies between our findings and those by Silk *et al.* (2017) may be due to different conditions in the vicinity of traps in the two studies. Trap placement can greatly affect the success of a trap (Dodds *et al.* 2010); baited traps in more open areas may attract more target insects than those placed in more wooded areas due to decreased obscuring of lure emissions. Alternatively, an interaction between host volatile sensation and trap type (shape) or trap colour could account for the discrepancy we see between these results (Campbell and Borden 2006). *Orchestes fagi* may exhibit a sensory hierarchy in which reactions to olfactory cues are decreased in the presence of certain visual stimuli (see Otálora-Luna *et al.* 2013). Perhaps septa placement in our prism traps affected our results. Septa were placed inside the mini-prism traps used in our study, making them less open to air movement than the lures on yellow sticky cards used by Silk *et al.* (2017), which could have decreased the release rate of the compound.

Every trap type used in our study captured large numbers of adult O. fagi in areas where O. fagi populations were previously recorded. Future studies should examine how beetle density affects trap performance. Additionally, practicality of each trap design is also an important point to consider. Panel traps are large and heavy relative to sticky traps; in areas with small trees, these traps were strung between two trees using a piece of rope. The salt water in the collection cup is prone to evaporation, so lengthy unaided deployments of this trap design are not feasible. However, the latter problem could be reduced using a different preservative such as recreational vehicle antifreeze (50% propylene glycol in water) or dry cup traps with insecticide strips (Miller and Duerr 2008). Additionally, about 10% of clips provided by the manufacturer to hold the pieces of the trap together broke after a few weeks of use, requiring twist tie wraps to tie the trap together. On the contrary, card traps are small, lightweight, portable, and disposable. They are easily deployed in the field and are durable enough to last an entire trapping season. The only downside to card traps is their small surface area. The surface area of this trap type limits its longevity in the field, since once the surface is obscured by catch it is no longer effective. Like card traps, prism traps are lightweight, durable, and can be deployed in trees of any size; prism traps have the added advantage of being reusable, requiring only the sticky tape to be replaced. Despite the similarities between prism traps and card traps, prism traps have a larger surface area (approximately three times larger) than card traps. This increased surface area theoretically increases the practical longevity of prism traps in the field.

This study sought to determine factors that enhanced trap performance by comparing the effects of trap colour, type, placement, and presence of a host volatile on trap catch. We were primarily interested in trap efficacy and, therefore, ignored any potential effects of pheromones, sounds, or visual cues from trapped insects on trap catches. In theory, chemical, auditory, and visual cues released by trapped *O. fagi* may have repelled or attracted subsequent *O. fagi* (Graham *et al.* 2012; Domingue *et al.* 2013). However, when monitoring traps are deployed in the field, trapped insects would produce these cues and so controlling for them in trap efficacy experiments would not help us understand the true efficacy of a trap in field conditions.

Overall, results from our experiments suggest that yellow prism traps are the best combination of trap type and colour used. Further research is necessary to determine whether yellow prism traps would have greater catch in the upper versus lower canopy. We found no increase in trap catch by baiting traps with 9-geranyl-*p*-cymene under the conditions of our study, but further studies to discern additional attractive host volatiles may lead to a more effective monitoring tool for *O. fagi.*

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