



Studies on the host selection of a weed biological control candidate facilitate pre-release environmental safety assessments

Bijay Subedi · Mark Schwarzländer · Sanford D. Eigenbrode ·
Bradley L. Harmon · Philip Weyl

Received: 24 October 2023 / Accepted: 13 March 2024 / Published online: 22 April 2024
© The Author(s) 2024

Abstract Pre-release host specificity testing can reliably predict the environmental safety of weed biological control agent (BCA) candidates but typically does not consider their host-finding behavior. Therefore, BCA candidates that do not utilize non-target plants in the field post-release, despite development on such plants in pre-release tests, may be prematurely disqualified for release. We addressed this issue with the seedpod weevil *Ceutorhynchus peyerimhoffi*, a BCA candidate for the invasive Eurasian mustard *Isatis tinctoria*. *Ceutorhynchus peyerimhoffi* weevils, both naïve and experienced, were tested for their responses to olfactory, visual, and combined olfactory and visual cues of *Braya alpina*, *Caulanthus*

heterophyllus, and the US federally listed *Boechera hoffmannii*. These responses were compared to *I. tinctoria* or control treatments in a modified Y-tube olfactometer set-up. Naïve and weevils with prior experience on *I. tinctoria* responded with attraction to olfactory, visual, and combined cues of *I. tinctoria*. In contrast, there was no attraction by either naïve or experienced weevils to non-target plant cues, except for attraction to *C. heterophyllus* combined cues by experienced weevils. Furthermore, visual cues of *B. alpina* and *B. hoffmannii* were repellent to experienced weevils, and olfactory cues of *B. alpina* were repellent to naïve weevils. We conclude that *C. peyerimhoffi* uses visual and olfactory cues to discriminate between its host plant *I. tinctoria* and North American non-targets. Behavioral host selection studies involving multimodal cues can provide a mechanistic explanation of host selection and complement environmental safety assessments of weed BCA candidates.

Handling Editor: Peter Mason.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10526-024-10255-4>.

B. Subedi · M. Schwarzländer · S. D. Eigenbrode ·
B. L. Harmon
Department of Entomology, Plant Pathology
and Nematology, University of Idaho, 875 Perimeter
Drive, MS 2339, Moscow, ID 83844-2339, USA

B. Subedi (✉)
Department of Entomology, Penn State University, 501
ASI Building, University Park, PA 16802, USA
e-mail: beejjus@gmail.com

P. Weyl
CABI, Rue des Grillons 1, 2800 Delémont, Switzerland

Keywords Host-finding behavior · Combined plant cues · *Ceutorhynchus peyerimhoffi* · *Isatis tinctoria* · Coleoptera

Introduction

Assessing the environmental safety of weed biological control agent (BCA hereafter) candidate species is a central aspect of classical biological weed control programs (Hinz et al. 2020). This is typically

accomplished through series of no-choice, choice, field cage, and open-field experiments (Schaffner 2001), prior to releasing them into a new environment (Heard 2000; Hinz et al. 2020). No-choice tests are used to assess the fundamental host range, which comprises plant species on which the larvae can complete their development. Choice tests (in cages, field cages, or open-field tests) are used to assess the realized host range, which comprises plant species that a BCA may use under natural conditions (Schaffner 2001). While the centrifugal phylogenetic approach (Wapshere 1974) is instrumental in guiding the selection of test plant species by suggesting that closely related plant species are more likely to serve as suitable alternative hosts for BCA development, studies such as Hinz et al. (2014) and Park et al. (2018) indicate that this method may have limitations in providing detailed insights into the host finding behavior of a candidate BCA. Host finding behavior, which encompasses the behavior exhibited by an insect herbivore during the initial stages of host-plant selection, often requires a more direct observational and experimental approach to capture the nuanced interactions between the herbivore and their potential host plants.

Insect herbivores use olfactory and visual cues from host plants to determine whether to accept or reject a plant during the host-finding stage of host selection (Bernays and Chapman 1994; Balkenius et al. 2009). The cue modalities used during host-finding include volatile organic compounds (VOCs) and foliar and floral colors or structures. The realized host range is typically a subset of the fundamental host range that only includes those species that are visited for potential colonization in the field. Species that are physiologically suitable to support development of a specialist herbivore BCA may be excluded from the realized host range because they lack cues to which the agent is responsive (Schaffner 2001; Schaffner et al. 2018). Understanding a BCA candidate's host-finding behavior could help explain discrepancies between its fundamental and realized host ranges (Heard 2000; Park et al. 2018) and aid in accurately predicting potential risks of non-target plant attack (Hinz et al. 2014, 2020).

While the host-finding behavior of herbivorous insects has been studied extensively (Knolhoff and Heckel 2014), few of these studies are conducted in the context of biological weed control and most

of those involve only one cue modality (either olfactory or visual cues) (Park et al. 2018; but see Andreas et al. 2009; Cossé et al. 2006 [olfactory cues]; Müller and Nentwig 2011; Reddy et al. 2009 [visual cues]). However, both cue modalities may play important roles during host-finding, and should therefore be considered during investigations, ideally simultaneously (Park et al. 2018; Subedi et al. 2023).

The Eurasian invasive herbaceous mustard *Isatis tinctoria* L. (Brassicaceae), native North American confamilial non-target plant species, and the BCA candidate, the seedpod feeding weevil *Ceutorhynchus peyerimhoffi* Hustache (Coleoptera: Curculionidae), provide a good study system to investigate the host-finding behavioral responses of a candidate BCA to visual and olfactory plant cues. In host range experiments, *C. peyerimhoffi* demonstrated a broad fundamental host range, as determined in no-choice tests (Weyl et al. 2017). However, when assessing the realized host range of *C. peyerimhoffi* in field cage choice tests, heavy attack (potential to kill a plant or reduce reproductive output substantially) was observed for *I. tinctoria* while other plant species, which were part of the fundamental host range, had only negligible attack (minor damage) in choice field cage tests (Weyl et al. 2017). The data suggest that host selection behavior underlies *C. peyerimhoffi*'s specialization on *I. tinctoria* but studies investigating potential mechanisms are lacking. The study system allows us to investigate the integration of multiple sensory modalities that insects use in the wild, which could lead to better understanding and more effective use of weed BCAs.

The aim of this study was to facilitate environmental safety assessments for *C. peyerimhoffi* through behavioral choice tests using visual and olfactory plant cues of critical native North American non-target plant species. We were interested in examining the host-finding behavior of *C. peyerimhoffi* with regard to those non-target species that supported the development of the weevil in previous traditional no-choice host range tests but escaped attack in choice field cages tests. We hypothesized that *C. peyerimhoffi* uses visual and/or olfactory plant cues to discriminate between *I. tinctoria* and non-target plant species attacked in no-choice larval development tests and that a combination of cues will allow greatest host discrimination. We tested whether *C. peyerimhoffi*

females respond to visual and olfactory cues of confamilial non-target plants in the absence of the Eurasian host, *I. tinctoria*, cues and whether *C. peyerimhoffi* females prefer visual and olfactory cues of *I. tinctoria* over the respective cues of native North American confamilial plant species.

Materials and methods

Study system

Isatis tinctoria (dyer's woad) is a Eurasian winter annual or biennial mustard that is invasive in the western USA (Gaskin et al. 2018). It is a declared noxious weed in 11 western US states (USDA NRCS 2023). The seedpod weevil *C. peyerimhoffi* is under consideration as a BCA for the control of *I. tinctoria* in the USA (Weyl et al. 2022). Females *C. peyerimhoffi* emerge during spring and feed on *I. tinctoria* inflorescence structures (Fig. S1). From late March to August, females typically lay eggs singly in developing *I. tinctoria* seedpods and the larvae feed on the seeds in the pod as they mature (Hinz et al. 2016). *C. peyerimhoffi* attack can reduce the seed output of *I. tinctoria* plants by 40–70% (Hinz et al. 2016).

Three North American non-target species confamilial to *I. tinctoria* were selected to study the pre-alignment host selection behavior of *C. peyerimhoffi*: *Braya alpina* Sternb. & Hoppe, *Boechera Hoffmannii* (Munz) Al-Shehbaz, and *Caulanthus heterophyllus* (Nutt.) Payson (see Table S1 for plant species details). These plant species were selected because they supported *C. peyerimhoffi* in no-choice developmental tests (Weyl et al. 2022). In addition, *B. hoffmannii* is restricted to a few locales in San Bernardino and San Diego Counties, California, USA, with the largest populations occurring on Santa Rosa and Santa Cruz Islands in Santa Barbara County, California (FNA 2023). The plant is a US federally listed threatened and endangered species in the USA (ECOS 2023).

All plant species were grown from seeds received from CABI Switzerland. Plants were grown in 3 l black plastic pots (diameter: 25 cm, height: 17 cm; T-pot Three, Stuewe and Sons, Inc, Tangent, OR, USA) using the following soil mix: one part sand and three parts Sunshine Mix No. 4 (Sun Gro

Horticulture Canada Ltd). All plants were watered as needed and maintained at 26 °C day and 15 °C night, and a L:D 16:8 photoperiod. Since the flowering phenostage of the plant species was required to conduct experiments with *C. peyerimhoffi*, 12-week-old plants, except *C. heterophyllus*, were vernalized in a cold room for 10–12 weeks at a L:D 12:12 photoperiod, and a constant temperature of 4 °C. As *C. heterophyllus* is an annual plant, it was only germinated once the other test plants were moved into the cooling room.

Adult *C. peyerimhoffi* used in this study were originally collected in Italy and laboratory reared at CABI Switzerland since 2008, with a single generation per year. We received adult *C. peyerimhoffi* from CABI Switzerland, in March 2019 ($n=300$), and maintained in a biological control quarantine laboratory at the University of Idaho, Moscow, ID, USA. The presence of ventral abdominal depressions was used to differentiate male weevils from females (Jordan et al. 1993). Twenty adults each, 15 females and five males, were maintained in transparent plastic cylinders (diameter: 11 cm, height: 15 cm, Semadeni AG, Ostermundigen, Switzerland) covered with a mesh lid in an environmental chamber (I-35 VL, Percival Mfg. Co., Boone, Iowa, USA), with fresh *I. tinctoria* flowers at 17 °C during the day and 8 °C at night, 60–70% RH, and a L:D 16:8 photoperiod. Since these weevils were allowed to feed on *I. tinctoria* flowers, they were classified as 'experienced' during choice tests. In April 2020, we received another shipment of *C. peyerimhoffi* larvae from CABI Switzerland. Larvae were kept at 5 °C in a rearing chamber (Percival Scientific Incubator, Model C-30, Percival Scientific, Inc., Perry, Iowa, USA) for emergence. A total of 67 adult *C. peyerimhoffi* emerged from these larvae. These weevils were separated into males and females as described above. Since these weevils had no feeding experience, they were classified 'naïve' and used immediately for choice tests.

For most experiments, each weevil was used only once to eliminate the possibility of learning or habituation affecting the outcomes. This was feasible due to the large number of weevils initially available. However, for some experiments conducted later, such as experiment 7, which compared the combined cues of test species versus *I. tinctoria*, limitations in the number of unused weevils available forced us

to re-use weevils that had previously been assayed. In these instances, we selected 13 unused and seven previously used weevils for each plant species to be tested in the experiment. The ratio of unused to previously used weevils were chosen to standardize the experiment among plant species within the constraints of our weevil population. To minimize confounding factors such as learning or habituation to *I. tinctoria* cues, we avoided weevils previously exposed to these cues. Furthermore, when re-using weevils, their selection was randomized to ensure no prior knowledge of their involvement in earlier tests, thereby safeguarding against inadvertent bias in the experimental design.

Collection of floral headspace volatiles for olfactory cues and flowering stems for visual cues

We used a portable volatile collection system (PVCS) for dynamic headspace VOC collections, which consists of a push pump to push the air into the volatile collection bag and a pull pump to pull out the equal amount of air, and flowmeters to regulate the amount of airflow (Park et al. 2019). Based on previous studies (e.g., Oluwafemi et al. 2011; Park et al. 2018, 2019), the concentration and airflow rates were chosen to balance the need for a realistic representation of natural VOC levels with practical considerations of the experimental design. For each collection, purified air (300 ml min^{-1}) was pushed using a Rena Air 400 pump (RENA, Chalfont, PA, USA) into a sealed sterilized polyvinyl bag ($14 \times 24 \text{ cm}$; Reynolds, Richmond, VA, USA) enclosing a flowering stem of a test plant at one end. The enclosed air, along with emitted floral headspace VOCs from the enclosed plastic bag, was pulled out at the same rate at the other end, passing through a VOC trap (40 mg Porapak Q, 80–100 mesh; Southern Scientific Inc. Micanopy, FL, USA) to absorb the emitted headspace VOCs (Park et al. 2019). Volatile collections were conducted for 3 h between (10:00 to 13:00) on sunny days for a group of six plants for a plant species with one empty bag as a control. Trapped VOCs were eluted with 200 μl of dichloromethane into screw cap vials (Supelco, Bellefonte, PA, USA) and stored at -80°C until further use. Flowering stems of individual test plants were cut and kept in 10 cm transparent aqua tubes (Syndicate Sales Inc., Kokomo, IN, USA) to use as a visual cue in visual choice tests.

Olfactometer choice tests

A modified glass Y-tube device (4 cm Y-stem, 12 cm arms, 2 cm internal diameter) was used to assess the weevil's behavioral responses to host and non-host visual and olfactory cues (see Fig. S2 for set-up). This set-up is adapted from Park et al. (2019). Purified air carrying eluted VOCs from a group of six plants is pushed into Y-tube arms from the end of each arm, and an equal amount of air is pulled out from the stem end. The Y-tube olfactometer was placed on a cylindrical plastic ring (internal diameter: 16 cm, external diameter: 20 cm; height: 5 cm; Fig. S2) with a transparent plastic top (Plastic Wrap, WinCo Foods, Boise, ID, USA). The cylindrical ring consisted of two openings (diameter: 3 cm) on the side that aligned with the arms of the Y-tube resting on it and introduced visual cues into the choice test (Fig. S2). After each replicate, the Y-tube was cleaned with 70% ethanol to remove residual olfactory cues and rotated 180° to avoid any positional effects (Park et al. 2019). In a sterilized 2 mm² filter paper, eluted VOCs (1 μl) were applied using a 10 μl manual syringe (Agilent Technologies, Sydney, Australia). Our olfactometer experiments proceeded under the assumption, based on Park et al. (2019), that insect responses to collected VOCs are comparable to responses to VOCs from whole-potted plants, without direct testing of this variable. Filter paper with eluted VOCs was then placed into a Tygon tube (R-3603, Saint-Gobain Corporation, Valley Forge, PA, USA) connected to the Y-tube arm (Fig. S2). The Rena® 400 pumps were used to push the purified air with eluted VOCs into the Y-tube's arm and pull it out through the stem end. Flowmeters (MR3000, Key Instruments, Hatfield, PA, USA) regulated the amount of airflow at 300 ml min^{-1} into each Y-tube arm. Both olfactory cues (VOCs) and visual cues (flowering stems) collected from six plants, each of the target and non-targets, were replaced every five replicates during the choice test. The Y-tube olfactometer arena was illuminated using a LED light (Jansjö® LED lamp, Inter Ikea System B. V., Delft, The Netherlands) by placing it directly above Y-tube (Fig. S2). The testing arena was enclosed in a double-layered rectangular box ($180 \times 90 \times 60 \text{ cm}$), with the inner layer being a white cloth to minimize visual distraction.

Experiments were conducted during the summer of 2019 and 2020 at the weevils' peak activity (i.e.,

feeding and oviposition). Since the females were kept together with males in a cylinder, they were assumed to have mated and ready for oviposition. All *C. peyerimhoffi* females were starved for 24 h prior to experiments to increase the responsiveness to cues (Defagó et al. 2016). Single *C. peyerimhoffi* females were introduced in the Y-tube at the weevil-release point (i.e., at the end of the Y-tube stem; Fig. S2) using forceps by briefly disconnecting the outlet hose at the stem end. A camera (Contour Roam 2, Contour Ins., and Seattle, Washington, USA) was used to record the weevil behavior. For each choice test, a replicate lasted 10 min. If the *C. peyerimhoffi* female did not cross a decision line (3 cm from the release point into the Y-tube arms; Fig. S2), the female was considered unresponsive, and was discarded from analyses. Approximately 88% of the weevils exhibited a response in the Y-tube olfactometer experiments, with this response rate being consistent across various experiments. For each choice test, the total time spent, i.e., time spent by weevils in each arm of the Y-tube, initial (first arm) choice, time taken to reach the decision line in the Y-tube arm (response time), and weevils' position in the Y-tube at the end of the 10 min recording period or final choice was recorded for each replicate. Total time spent was used to measure the strength of a female's preference for cues offered. Initial choice (choosing the first arm) and response time variables were used to measure the weevil's ability and agility to discriminate between the offered cues. Final choice was noted to explain the weevil's ultimate preference for the offered cues. There were three possible outcomes: indifference, repellence, and attraction. For this study, we defined indifference (a response where the weevils did not exhibit a statistically significant preference for or against the test stimuli compared to the control treatments) as occurring when a plant's VOCs and/or visual cues were not more or less preferred by *C. peyerimhoffi* than control cues in choice test (purified air and/or absence of a visual cue; Martini et al. 2015). Attraction occurred if plant VOCs and/or visual cues were significantly greater over the control treatment and repellence occurred if *C. peyerimhoffi* preferred control treatments over test plant cues (Vet et al. 1983). For each trial, the sample size ranged between 10 and 25 individual females. All choice tests were conducted in the biological control quarantine laboratory between 9:00 and 14:00 h,

aligning with the diurnal activity patterns of the weevils as observed in the field (Weyl, unpublished data). The tests were carried out at a controlled temperature of 21 °C and 50% RH. At the beginning of experimentation, a blank test with purified air was conducted to test for orientation bias between the Y-tube arms.

Olfactometer choice experiments were conducted with experienced females in the summer of 2019 and naïve weevils in the summer of 2020. The first three experiments focused on naïve weevils and were conducted with all test plant species, except for *B. hoffmannii*, which was excluded due to the unavailability of its flowering phenostage. Experiment 1 compared olfactory plant cues (eluted VOCs) to purified air. Experiment 2 assessed visual plant cues (flowering stems) against empty arms, and experiment 3 evaluated weevil responses to a combined (olfactory and visual) plant cues. These experiments were replicated in experiments 4, 5, and 6 with experienced weevils. Experiment 7, conducted only with experienced weevils, explored their preference for combined plant cues of non-target species versus those of *I. tinctoria*, presenting these cues in a modified Y-tube device to understand the weevils' relative preference in the presence of *I. tinctoria* cues. All experiments employed a Y-tube set-up, with visual cues presented under one arm and olfactory cues pushed through one arm, as detailed above (Fig. S3).

For the experiments with experienced weevils, the trial sequence was structured as follows: (1) Olfactory cues versus control (experiment 4), with one day dedicated to each of the four plant species under study for a total of four days; (2) visual cues versus control (experiment 5), conducted over the subsequent four days, again with one plant species tested each day; (3) combined cues versus control (experiment 6), again conducted during the subsequent four days; and (4) Test plant species combined cues versus *I. tinctoria* combined cues (experiment 7), conducted during three days since only three plant species were tested. For the experiment with naïve weevils, we replicated the sequence used for experienced weevils, completing this over a span of three days.

Statistical analysis

Statistical analyses were conducted using R version 4.3.1 (R Core Team 2024), with a focus on the key variables: time spent, initial choice, final choice, and response time. To standardize the weevil time spent response, we measured the proportion of time spent in each arm of the Y-tube, excluding the time spent in the weevil-release area.

Using the ‘cor’ function from the ‘stats’ package, pairwise correlations were calculated to assess relationships among the variables. Data were grouped by experimental parameters, including plant species (*I. tinctoria*, *B. alpina*, *B. hoffmannii*, *C. heterophyllus*), type of experiment, and weevil status (naïve or experienced). This analysis pinpointed a moderate and consistent correlation between initial and final choice variables (Table S2). Consequently, we focus primarily on initial choice, relegating final choice details to supplementary materials (Fig. S4, Table S3). Additionally, response time, which displayed negligible correlations with other variables but lacked significant treatment differences across most experiments, is reported in supplementary materials (Table S4).

A critical examination of the random effects in the Generalized Linear Mixed Model (GLMM; ‘glmmTMB’ package; Brooks et al. 2017) for time spent and initial choice indicated an inconsequential variance attributed to the ‘replicate’ or ‘repeated use of weevils.’ This lack of significant variability led to the exclusion of random effects, leading to the use of Generalized Linear Models (GLMs; ‘lme4’ package; Bates et al. 2015). A Generalized Linear Model (GLM) with a Gaussian error distributions was utilized to analyze time spent, followed by a likelihood ratio test (ANOVA; ‘car’ package; Fox and Weisberg 2019). For initial choice, we conducted a GLM with Binomial error distributions and logit link function for different plant species in each experiment, where Wald tests evaluated the significance of individual predictors, and the Z-value was determined as the ratio of the estimate to its SE. These models included ‘treatment’ as the explanatory variable, discerning between cues from plant species versus control (purified air/empty arm), or *I. tinctoria* versus non-target species cues. The outcomes from these GLMs are presented graphically in the results section, with time spent data depicted as proportions

and initial choice data as percentages. To determine their relative influence of plant cues on time spent, behavioral responses of female *C. peyerimhoffi* were analyzed using a GLM with Gamma error distributions and inverse link function, contrasting olfactory, visual, and combined cues. Post-hoc comparisons of cue modalities were executed using estimated marginal means, facilitating a detailed assessment of individual versus combined cue effects. For all tests, P-values < 0.05 were considered significant.

Results

Experiment 1: olfactory cues versus purified air with naïve weevils

For time spent, naïve *C. peyerimhoffi* females spent more time in the arm with *I. tinctoria* volatiles than those containing purified air ($\chi^2_{(1)} = 37.815$, $P < 0.0001$; Fig. 1a). In contrast, females spent more time in arms with purified air compared to *B. alpina* olfactory cues ($\chi^2_{(1)} = 19.606$, $P < 0.0001$; Fig. 1a), and there was no significant difference for the time spent in arms filled with purified air or *C. heterophyllus* volatiles ($\chi^2_{(1)} = 0.076$, $P = 0.782$; Fig. 1a). Weevils did not prefer any tested species for initial choice (*I. tinctoria*: $Z = 1.754$, $P = 0.080$, *B. alpina*: $Z = 1.829$, $P = 0.067$, and *C. heterophyllus*: $Z = 0.301$, $P = 0.763$; Fig. 1b).

Experiment 2: visual test plant cues (flowering stems) versus empty arms with naïve females

Response of naïve *C. peyerimhoffi* females differed for time spent between visual cues of *I. tinctoria* and the empty arm ($\chi^2_{(1)} = 4.182$, $P = 0.0409$; Fig. 1c). For all other test plant species, time spent by naïve *C. peyerimhoffi* females were not significantly different from the empty arm (*B. alpina*: $\chi^2_{(1)} = 0.129$, $P = 0.719$, and *C. heterophyllus*: $\chi^2_{(1)} = 1.548$, $P = 0.213$; Fig. 1c). Weevils were indifferent to flowering stems of all tested species for initial choice (*I. tinctoria* ($Z = 1.648$, $P = 0.099$), *B. alpina* ($Z = 0$, $P = 1$), and *C. heterophyllus* ($Z = 0$, $P = 1$); Fig. 1d).

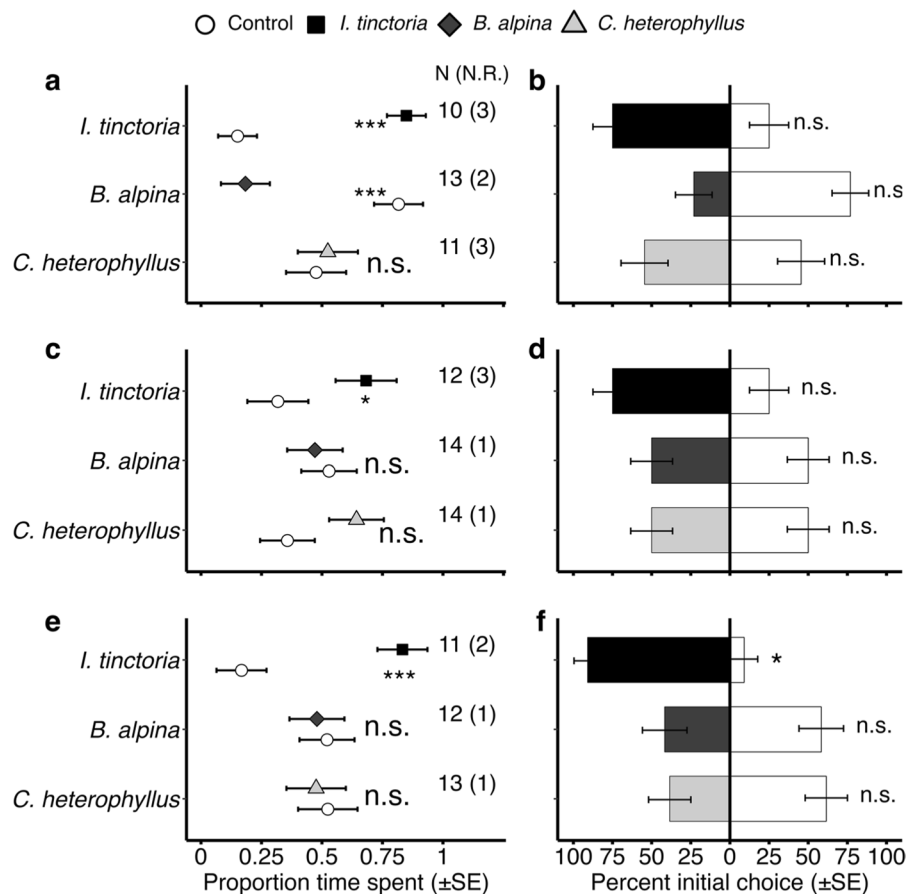


Fig. 1 Proportion of time spent (mean ± SE) and percent initial choice (mean ± SE) by naïve *Ceutorhynchus peyerimhoffi* in a modified Y-tube choice tests with olfactory cues (time spent: **a**, initial choice: **b**), visual cues (time spent: **c**, initial choice: **d**), combined cues (time spent: **e**, initial choice: **f**) of native North American confamilial plant species and *Isatis tinctoria* against control (purified air and/or empty arm). Olfactory cues were derived from eluted floral volatiles, with flow-

ering stems providing visual cues. Generalized Linear Model of individual experiments (* $P < 0.05$, *** $P < 0.001$; n.s. not significant ($P > 0.05$)). Significance (*, ***) are for the null hypothesis of 50% time spent in or initial choice for the treatment (or control) arm. Numbers next to each right bar on time spent indicate the sample size for that experiment (N Responders, N.R. Non responders (discarded from analysis))

Experiment 3: combined olfactory and visual test plant cues versus control with naïve females

Naïve weevils preferred combined olfactory and visual cues of *I. tinctoria* over purified air and empty arms ($\chi^2_{(1)} = 20.715$, $P < 0.0001$; Fig. 1e), but there was no difference in time spent of females to combined cues of *B. alpina* ($\chi^2_{(1)} = 0.067$, $P = 0.795$; Fig. 1e) and *C. heterophyllus* ($\chi^2_{(1)} = 0.077$, $P = 0.780$; Fig. 1e), and control treatments, respectively. Naïve weevils chose *I. tinctoria* combined cues over the control arm ($Z = 2.195$, $P = 0.002$; Fig. 1f). No significant preference for combined cues of *B. alpina*

($Z = 0.575$, $P = 0.566$; Fig. 1f) and *C. heterophyllus* ($Z = -0.824$, $P = 0.41$; Fig. 1f) was found for initial choice.

Responses of naïve female weevils to combined olfactory and visual cues of tested species was not different from their respective individual olfactory and visual cues for time spent ($P > 0.05$; Table S5). In a test of the relative strength of cues, no difference in naïve *C. peyerimhoffi* response between olfactory cues, visual cues, and combined olfactory-visual cues was detected ($P > 0.05$; Table S6).

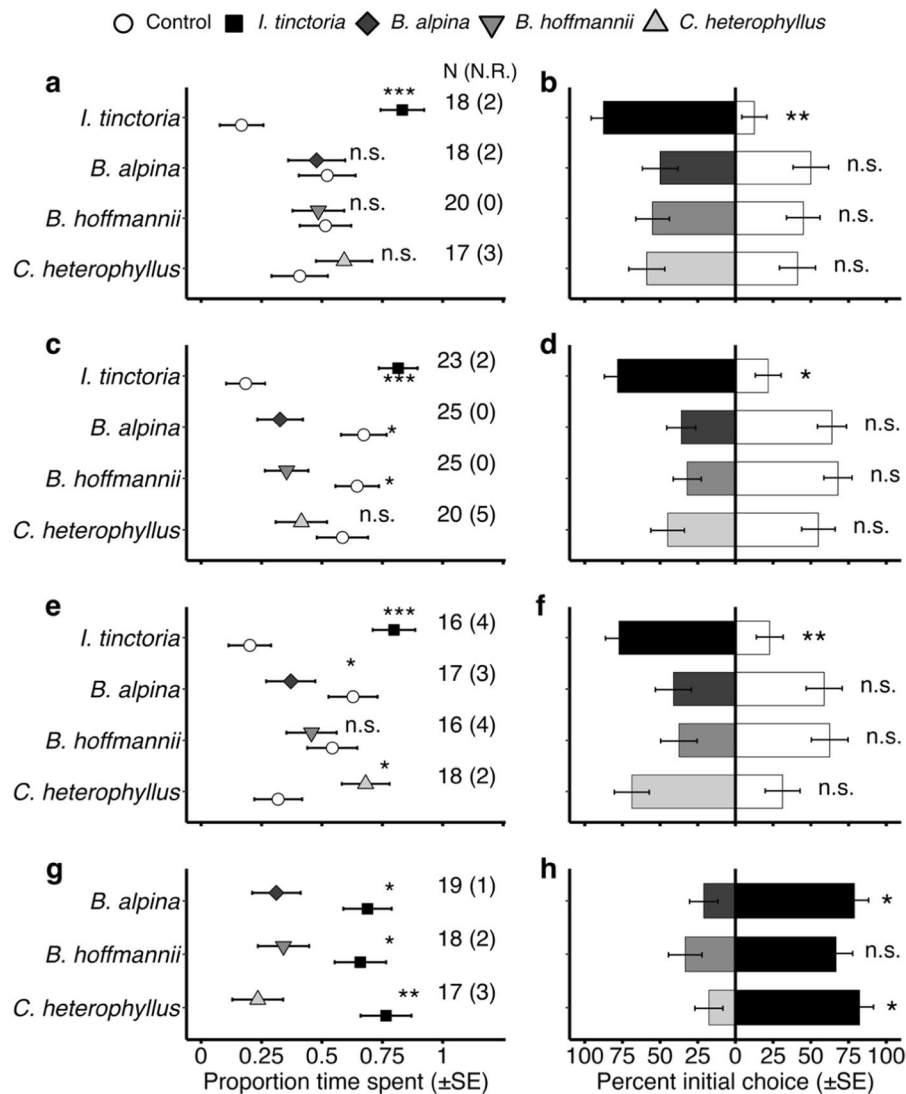


Fig. 2 Proportion of time spent (mean $\pm$ SE) and percent initial choice (mean $\pm$ SE) by experienced *Ceutorhynchus peyerimhoffi* in a modified Y-tube choice tests with olfactory cues (time spent: **a**, initial choice: **b**), visual cues (time spent: **c**, initial choice: **d**), combined cues (time spent: **e**, initial choice: **f**) of test plant species against control (purified air and/or empty arm, white bars), and proportion of time spent (mean $\pm$ SE) and percent initial choice (mean $\pm$ SE) by experienced *C. peyerimhoffi* in a modified Y-tube choice tests with combined cues of native North American confamilial plant species against *Isatis*

tinctoria cues (time spent: **g**, initial choice: **h**). Olfactory cues were derived from eluted floral volatiles, with flowering stems providing visual cues. Generalized Linear Model of individual experiments (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; n.s.: not significant ($P > 0.05$)). Significance (*, **, ***) are for the null hypothesis of 50% time spent in or initial choice for the treatment (or control) arm. Numbers next to each right bar on time spent indicate the sample size for that experiment (N Responders, N.R. Non responders (discarded from analysis))

Experiment 4: olfactory test plant cues versus purified air with experienced females

Experienced *C. peyerimhoffi* females spent more time in the arm with *I. tinctoria* volatiles than with

purified air ($\chi^2_{(1)} = 27.197$, $P < 0.0001$; Fig. 2a). There was no preference for time spent between olfactory cues of non-target plant species and purified air (*B. alpina*: $\chi^2_{(1)} = 0.069$, $P = 0.793$, *B. hoffmannii*: $\chi^2_{(1)} = 0.038$, $P = 0.845$, and *C. heterophyllum*:

$\chi^2_{(1)}=1.250$, $P=0.263$; Fig. 2a). When VOCs from plants were presented in the Y-tube against purified air, experienced female *C. peyerimhoffi* preferred *I. tinctoria* for initial choice ($Z=2.545$, $P=0.011$; Fig. 2b) and were indifferent to all other test species (*B. alpina*: $Z=0$, $P=1$, *B. hoffmannii*: $Z=0.446$, $P=0.655$, and *C. heterophyllus*: $Z=0.724$, $P=0.469$; Fig. 2b).

Experiment 5: visual plant cues versus an empty arm with experienced females

Experienced female *C. peyerimhoffi* spent more time in the arm with *I. tinctoria* flowering sprigs compared to the empty control arm ($\chi^2_{(1)}=30.867$, $P<0.0001$; Fig. 2c). There was no difference in time spent between arms with *C. heterophyllus* visual cues and empty control arms ($\chi^2_{(1)}=0.412$, $P=0.520$; Fig. 2c). In contrast, weevils spent more time in the empty control arms than those with visual cues of *B. alpina* ($\chi^2_{(1)}=6.741$, $P=0.009$; Fig. 2c) and *B. hoffmannii* ($\chi^2_{(1)}=5.247$, $P=0.022$; Fig. 2c), respectively. Experienced females preferred *I. tinctoria* flowering stems over the empty arm for initial choice ($Z=2.832$, $P=0.005$; Fig. 2d) and were indifferent to flowering stems of other test species (*B. alpina*: $Z=1.381$, $P=0.167$, *B. hoffmannii*: $Z=1.758$, $P=0.079$, and *C. heterophyllus*: $Z=-0.446$, $P=0.655$; Fig. 2d).

Experiment 6: combined olfactory and visual cues versus control treatments with experienced females

Experienced females preferred olfactory and visual cues of *I. tinctoria* ($\chi^2_{(1)}=32.576$, $P<0.0001$; Fig. 2e) and those of *C. heterophyllus* ($\chi^2_{(1)}=6.694$, $P=0.009$; Fig. 2e) over purified air and empty arms for the time spent. There was no difference in the time spent for combined cues of *B. alpina* ($\chi^2_{(1)}=3.222$, $P=0.072$; Fig. 2e) and *B. hoffmannii* ($\chi^2_{(1)}=0.343$, $P=0.557$; Fig. 2e) against the control. For initial choice, females preferred *I. tinctoria* ($Z=2.622$, $P=0.009$; Fig. 2f) over an empty arm and were indifferent to *B. alpina* ($Z=0.724$, $P=0.469$; Fig. 2f), *B. hoffmannii* ($Z=0.989$, $P=0.323$; Fig. 2f), and *C. heterophyllus* ($Z=1.462$, $P=0.144$; Fig. 2f).

The response of the weevils to combined olfactory and visual cues did not differ from the individual olfactory and visual cues for *I. tinctoria* for time spent ($P>0.05$; Table S5). In a test of the relative strength of cues, no difference in experienced *C. peyerimhoffi* response between olfactory cues, visual cues, and combined olfactory-visual cues was detected for time spent ($P>0.05$; Table S6).

Experiment 7: combined olfactory and visual cues of non-target species versus combined cues of *I. tinctoria*

In choice tests for olfactory and visual plant cues of confamilial non-target plant species against those of *I. tinctoria*, experienced female weevils preferred *I. tinctoria* over all non-target species tested based on time spent in arms (*B. alpina*: $\chi^2_{(1)}=7.079$, $P=0.007$, *B. hoffmannii*: $\chi^2_{(1)}=4.473$, $P=0.034$, and *C. heterophyllus*: $\chi^2_{(1)}=12.747$, $P=0.004$; Fig. 2g). For initial choice, weevils preferred *I. tinctoria* over *B. alpina* ($Z=2.349$, $P=0.019$; Fig. 2h) and *C. heterophyllus* ($Z=-2.421$, $P=0.015$; Fig. 2h), but not over *B. hoffmannii* ($Z=1.386$, $P=0.166$; Fig. 2h).

Discussion

In this study, we investigated the host-finding behavior of the biological control agent (BCA) candidate *C. peyerimhoffi* in relation to its Eurasian field host *I. tinctoria* and three native North American confamilial non-target plant species (*B. alpina*, *B. hoffmannii*, and *C. heterophyllus*). We conducted choice tests to assess the weevil's responses to both visual and olfactory plant cues in a modified Y-tube olfactometer, measuring four variables (time spent, initial choice, final choice, and response time). Our results indicate that *C. peyerimhoffi* females prefer *I. tinctoria* plant cues while exhibiting no preference for either visual or olfactory cues, or their combination, in almost all tests with the non-target species. Most responses to non-targets during choice tests indicated indifference or repulsion, except experienced *C. peyerimhoffi* females that were attracted to the combined olfactory and visual cues of *C. heterophyllus*. Our results suggest that the host

range of *C. peyerimhoffi* is limited by its host-finding behavior, similar to other biological control agents (Chu et al. 2019; Kafle 2016). Our data are consistent with the concept that the realized host range of a BCA candidate species is typically narrower than its fundamental host range (Schaffner 2001).

The data indicate that *C. peyerimhoffi* females are attracted to both olfactory and visual cues of *I. tinctoria*, regardless of prior feeding experience. Both olfactory and visual cues alone were sufficient to elicit attraction in both naïve and experienced females, suggesting that *C. peyerimhoffi* utilizes both olfactory and visual cues in host finding and discrimination. Additionally, in choice tests between *I. tinctoria* and non-target species with combined cues, experienced *C. peyerimhoffi* females strongly preferred their Eurasian field host *I. tinctoria* over native North American species tested, suggesting that the weevil can discriminate between *I. tinctoria* and all three tested native North American plant species. This finding could explain the observed differences in the *C. peyerimhoffi* attack between *I. tinctoria* and each test plant species in choice field cage tests (see Weyl et al. 2017). Our findings support prior results of traditional host range investigations of *C. peyerimhoffi* and suggest that visual and olfactory cues are not only crucial in *C. peyerimhoffi*'s host finding and acceptance but that they also contribute to its narrow realized host range.

Indifferent and repellant responses of naïve and experienced *C. peyerimhoffi* females to various plant cues of *B. alpina* and *B. hoffmannii* suggest that the weevil is unable to identify these plant species as potential hosts in the field during pre-alignment stages of host finding. Olfactory cues are an essential component in host finding (Bernays and Chapman 1994) as they can provide reliable information regarding host-plant quality (host suitability, nutritional quality; Tasin et al. 2011). Similarly, Reeves (2011) suggests that host and non-host visual cues are perceptible and reliably used by herbivores. Therefore, the observed indifference or repellent response of *C. peyerimhoffi* to olfactory and visual cues of *B. alpina* and *B. hoffmannii* suggest these species are non-hosts (Tasin et al. 2011) that go undetected or even repel the weevil in the field (Byers et al. 2004; Deletre et al. 2016). Specialist herbivores with narrow host ranges, such as *C. peyerimhoffi*, may use olfactory (Tasin et al. 2011) and/or visual cues

(Stenberg and Ericson 2007) to efficiently distinguish suitable hosts from non-hosts to maintain their host fidelity.

In choice tests, both naïve and experienced *C. peyerimhoffi* females showed no attraction to individual olfactory, visual or combined cues of *C. heterophyllus* for both initial choice and final choice parameters. However, experienced females preferred combined cues over the control based on the amount of time spent in the Y-tube arm, indicating that prior feeding experience on *I. tinctoria* allows *C. peyerimhoffi* to identify *C. heterophyllus* as a potential host. The reason for this incongruent pattern of preference is unclear. Both naïve and experienced *C. peyerimhoffi* tended to prefer olfactory cues of *C. heterophyllus* over the control based on time spent in the olfactometer arm, suggesting that the floral headspace volatile profile of *C. heterophyllus* includes at least one potentially attractive VOC. Previous research on *Mogulones crucifer* Pallas (Coleoptera: Curculionidae) has shown that its attraction to *C. officinale* can be triggered by a single bioactive VOC (methyl isovalerate) (Kafle 2016). However, the lack of attraction based on both initial and final choice suggests that *C. heterophyllus* cues may present a broken or incomplete signal to which the weevils respond inconsistently. Furthermore, we found that *C. peyerimhoffi* displayed a greater preference for combined visual and olfactory cues of its host plant *I. tinctoria* over those of *C. heterophyllus*. This suggests that the floral headspace volatile profile of *I. tinctoria* contains attractive VOCs not present in the profile of *C. heterophyllus*. Weevils may show increased sensitivity and fidelity to complete cues, leading to more consistent responses (Li et al. 2017), underscoring the importance of complete and reliable information during decision-making.

In our experiments we used yellow-flowered *C. heterophyllus*, but it has been reported to have different inflorescence morphotypes with varying sepal and petal colors (Brassibase 2023). Because our study tested one morphotype of *C. heterophyllus*, namely the one most resembling *I. tinctoria*, our results may not reflect responses by the weevil to the morphotype diversity of the species. This limitation could explain the observed attraction of *C. peyerimhoffi* to the combined visual and olfactory cues of *C. heterophyllus* in our choice tests (Lyu et al. 2021). Additionally,

metabolic pathways for floral color and volatiles in inflorescences may be shared, resulting in the correlation among these traits. Selection of specific floral volatile compounds may also be due to the direct selection of floral color (Zvi et al. 2008). Therefore, the differing inflorescence morphotypes of *C. heterophyllus* may not only differ in floral coloring but also volatile profiles. For example, Ascrizzi and Flamini (2020) found that the floral volatile profile of *Iris lutescens* Lam. (Iridaceae) differed between two inflorescence morphotypes growing in serpentine soil. Different morphotypes of *C. heterophyllus* may produce different floral reflectance and VOC profiles, potentially leading to different behavioral responses from *C. peyerimhoffi* than observed in our study.

Ideally, experiments should be conducted using naïve insects to exclude potentially confounding factors like female age or gravidity that could affect test results (Thompson 1988). Here, that was not possible because the availability and logistics of obtaining weevils prevented the use of naïve individuals.

Our experiments show that *C. peyerimhoffi* utilizes both visual and olfactory cues to distinguish its host plant, *I. tinctoria*, from three native North American non-target confamilial species. This utilization of host-finding cues may help maintain the weevil's host fidelity. Also, these results highlight the importance of including both cue modalities in behavioral experiments used for environmental safety assessments. This information can assist in differentiating the realized and fundamental host range of *C. peyerimhoffi* as assessed in traditional host range investigations. Our findings also suggest that *C. peyerimhoffi* is unlikely to cause any non-target damage to *B. alpina* or the endangered *B. hoffmannii* post-release. While the results of our experiments on pre-alignment host-finding are encouraging, it should be noted that post-alignment cues can also contribute to host selection by weed biological control agents (Das et al. 2019) limiting their realized host range. Studies like ours, as well as those conducted by Park et al. (2018), Fung et al. (2021), and Subedi et al. (2023), can provide insight into the mechanisms underlying host selection among biological control agent candidates and complement traditional methods of testing host specificity. Further research

linking host selection behavior and traditional field testing of BCAs can aid in designing effective pre-release field studies and improve the accuracy of conclusions drawn from these studies.

Acknowledgements We want to thank Ying Wu (University of Idaho, USA) for her assistance with instrumentation and Dr. Bill Price (University of Idaho, USA) and Alice Pessina for their advice on statistical analyses. CABI Switzerland kindly provided seeds for all test plant species and *Ceutorhynchus peyerimhoffi* weevils. We would especially like to thank Dr. Harriet Hinz (CABI) for inspiring this research and for discussions throughout the study.

Author contributions MS and BS conceived and designed the experiments. BS and BLH propagated plants and performed the experiment. BS, SDE, and MS analyzed data. MS, BS, SDE, and PW wrote the manuscript.

Funding Financial support for this project was provided by the USDI, Bureau of Land Management, CESU Agreement L19AC00080 to MS. This is a publication of the Idaho Agricultural Experimental Station.

Data availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no competing or conflicts of interest.

Ethical approval This paper does not contain any studies with human participants or animals performed by any of the authors.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Andreas J, Schwarzländer M, Ding H, Eigenbrode SD (2009) Post-release non-target monitoring of *Mogulones cruciger*, a biological control agent released to control *Cynoglossum officinale* in Canada. In: Julien MH, Sforza

- R, Bon MC, Evans HC, Hatcher PE, Hinz HL, Rector BG (eds) Proceedings of the 12th international symposium on biological control of weeds. CAB International, Wallingford, pp 75–82
- Ascrizzi R, Flamini G (2020) *Iris lutescens* on serpentine soil: volatile emission profiles in different organs of its two colour morphs. *Plant Biosyst* 155:406–414
- Balkenius A, Bisch-Knaden S, Hansson B (2009) Interaction of visual and odour cues in the mushroom body of the hawkmoth *Manduca sexta*. *J Exp Biol* 212:535–541
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48
- Bernays EA, Chapman RF (1994) Host plant selection by phytophagous insects. Chapman and Hall, New York
- BrassiBase (2023) BrassiBase. <https://brassibase.cos.uni-heidelberg.de/> Accessed 26 August 2022
- Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J* 9:378–400
- Byers J, Zhang Q, Birgersson G (2004) Avoidance of nonhost plants by a bark beetle, *Pityogenes bidentatus*, in a forest of odors. *Naturwissenschaften* 91:215–219
- Chu S, Cong S, Li R, Hou Y (2019) Host range of *Herpetogramma basalis* (Lepidoptera: Crambidae), a biological control agent for the invasive weed *Alternanthera philoxeroides* (Centrospermae: Amaranthaceae) in China. *J Insect Sci* 19(6):1–7
- Cossé AA, Bartelt RJ, Zilkowski BW, Bean DW, Andress ER (2006) Behaviorally active green leaf volatiles for monitoring the leaf beetle, *Diorhabda elongata*, a biocontrol agent of saltcedar, *Tamarix* spp. *J Chem Ecol* 32:2695–2708
- Das S, Koner A, Barik A (2019) A beetle biocontrol agent of rice-field weeds recognizes its host plants by surface wax long-chain alkanes and free fatty acids. *Chemoecology* 29:155–170
- Defagó MT, Videla M, Valladares G (2016) To smell you better: prior food deprivation increases herbivore insect responsiveness to host plant odor cues. *J Insect Behav* 29:527–534
- Deletre E, Schatz B, Bourguet D, Chandre F, Williams L, Ratnadass A, Martin T (2016) Prospects for repellent in pest control: current developments and future challenges. *Chemoecology* 26:127–142
- ECOS (2023) Environmental Conservation Online System. U.S. Fish & Wildlife Service. <https://ecos.fws.gov/ecp/species/5909> Accessed 04 January 2023
- FNA (2023) Flora of North America. http://beta.floranorthamerica.org/Volume_17 Accessed 04 August 2022
- Fox J, Weisberg S (2019) An R companion to applied regression. SAGE, Thousand Oaks
- Fung JM, Nepal K, Kaffle BD, Eigenbrode SD, Schwarzländer M (2021) Locomotory responses to olfactory cues during host-finding can inform environmental safety assessments of biological weed control agents. *Entomol Exp Appl* 169:758–771
- Gaskin JF, Schwarzländer M, Gibson RD, Simpson H, Marshall DL, Gerber E, Hinz HL (2018) Geographic population structure in an outcrossing plant invasion after centuries of cultivation and recent founding events. *AoB Plants* 10:ply020
- Heard TA (2000) Concepts in insect host-plant selection behavior and their application to host specificity testing. In: van Driesche RG, Heard T, McClay A, Reardon R (eds) Proceedings host specificity testing of exotic arthropod biological control agents: the biological basis for improvement in safety. US Forest Service, Forest Health Technology Enterprise Team, Morgantown, pp 1–10
- Hinz HL, Schwarzländer M, Gassmann A, Bouchier RS (2014) Successes we may not have had: a retrospective analysis of selected weed biological control agents in the United States. *Invas Plant Sci Mana* 7:565–579
- Hinz HL, Closca C, Castellan I, Heijs W, Morelon S (2016) Biological control of dyer's woad, *Isatis tinctoria*, annual report 2015. CABI, Delemont
- Hinz HL, Winston RL, Schwarzländer M (2020) A global review of target impact and direct nontarget effects of classical weed biological control. *Curr Opin Insect Sci* 38:48–54
- Kaffle B (2016) Olfaction mediated host selection in a specialist weevil used for biological control of an invasive plant. MSc Thesis, Department of Entomology, Plant Pathology and Nematology. University of Idaho, Moscow, ID, USA
- Knolhoff LM, Heckel DG (2014) Behavioral assays for studies of host plant choice and adaptation in herbivorous insects. *Annu Rev Entomol* 59:263–278
- Jordan T, Schwarzländer M, Tosevski I, Freese A (1993) *Ceutorhynchus cruciger* Herbst (Coleoptera: Curculionidae): a candidate for the biological control of hound's-tongue (*Cynoglossum officinale* L., Boraginaceae) in Canada. IIBC, unpublished final report.
- Li WZ, Teng XH, Zhang HF, Liu T, Wang Q, Yuan G, Guo XR (2017) Comparative host selection responses of specialist (*Helicoverpa assulta*) and generalist (*Helicoverpa armigera*) moths in complex plant environments. *PLoS ONE* 12(2):e0171948
- Lyu F, Hai XX, Wang ZG (2021) Green-colored paperboard enhances the Asian longhorned beetle response to host plant odor cues. *J Pest Sci* 94:1345–1355
- Martini X, Hughes MA, Smith JA, Stelinski LL (2015) Attraction of redbay ambrosia beetle, *Xyleborus glabratus*, to leaf volatiles of its host plants in North America. *J Chem Ecol* 41:613–621
- Müller E, Nentwig W (2011) How to find a needle in a haystack—host plant finding of the weevil *Ceratopion onopordi*. *Entomol Exp Appl* 139:68–74
- Oluwafemi S, Bruce TJ, Pickett JA, Ton J, Birkett MA (2011) Behavioral responses of the leafhopper, *Cicadulina storeri* China, a major vector of maize streak virus, to volatile cues from intact and leafhopper-damaged maize. *J Chem Ecol* 37:40–48
- Park I, Eigenbrode SD, Cook SP, Harmon BL, Hinz HL, Schaffner U, Schwarzländer M (2018) Examining olfactory and visual cues governing host-specificity of a weed biological control candidate species to refine pre-release risk assessment. *BioControl* 63:377–389
- Park I, Schwarzländer M, Hinz HL, Schaffner U, Eigenbrode SD (2019) A simple approach to evaluate behavioral responses of insect herbivores to olfactory and visual cues simultaneously: the double stacked y-tube device and

- portable volatile collection system. *Arthropod-Plant Interact* 13:139–149
- R Core Team (2024) R: a language and environment for statistical computing. R foundation for statistical computing. Vienna, Austria. <https://www.r-project.org/>
- Reddy GVP, Cruz ZT, Braganza N, Muniappan R (2009) Response of *Melitita oedipus* (Lepidoptera: Sesiidae) to visual cues is increased by the presence of food source. *J Econ Entomol* 102:127–132
- Reeves JL (2011) Vision should not be overlooked as an important sensory modality for finding host plants. *Environ Entomol* 40:855–863
- Schaffner U (2001) Host range testing of insects for biological weed control: how can it be better interpreted? *BioScience* 51:951–959
- Schaffner U, Smith L, Cristofaro M (2018) A review of open-field host range testing to evaluate nontarget use by herbivorous biological control candidates. *BioControl* 63:405–416
- Stenberg JA, Ericson L (2007) Visual cues override olfactory cues in the host-finding process of the monophagous leaf beetle *Altica engstroemi*. *Entomol Exp Appl* 125:81–88
- Subedi B, Schwarzländer M, Eigenbrode SD, Harmon BL, Weyl P (2023) Understanding the host finding behavior of a biological weed control candidate specialist as a contribution to pre-release risk assessments. *Entomol Exp Appl* 171:943–953
- Tasin M, Betta E, Carlin S, Gasperi F, Mattivi F, Pertot I (2011) Volatiles that encode host-plant quality in the grapevine moth. *Phytochemistry* 72:1999–2005
- Thompson JN (1988) Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomol Exp Appl* 47:3–14
- USDA NRCS (2023) The PLANTS database. <http://plants.usda.gov> Accessed 04 August 2022
- Vet LE, Lenteren JV, Heymans M, Meelis E (1983) An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. *Physiol Entomol* 8:97–106
- Wapshere AJ (1974) A strategy for evaluating the safety of organisms for biological weed control. *Ann Appl Biol* 77:201–211
- Weyl P, Cloșca C, Hinz HL, Vidović B, Petanović R, Cristofaro M, Dieudonné E, Vasilev L (2017) Biological control of dyer's woad, *Isatis tinctoria*, annual report 2016. CABI, Delemont
- Weyl P, Cloșca C, Marini F, Hinz HL (2022) Biological control of dyer's woad, *Isatis tinctoria*, annual report 2021. CABI, Delemont
- Zvi MM, Negre-Zakharov F, Masci T, Ovadis M, Shklarman E, Ben-Meir H, Tzfira T, Dudareva N, Vainstein A (2008) Interlinking showy traits: co-engineering of scent and colour biosynthesis in flowers. *Plant Biotechnol J* 6:403–415

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Bijay Subedi is a graduate student in the Department of Entomology, Plant Pathology and Nematology at the University of Idaho, USA. He works on classical biological control of invasive weeds including host range testing and nontarget risk assessment.

Mark Schwarzländer is a professor of entomology at the University of Idaho, USA and associate director of Center for Research on Invasive Species. His research focuses on all aspects of classical biological control of weeds of Eurasian origin in western USA grasslands.

Sanford D. Eigenbrode is a university distinguished professor of entomology and an affiliate faculty in the Center for Research in Invasive Species at the University of Idaho, USA. His research focuses are insect-plant interactions, landscape agricultural ecology, climate change, the chemical ecology of plant viruses and their vectors, and weed biological control. His interests also include the science and practice of cross-disciplinary collaboration.

Bradley L. Harmon is a research support scientist in the Department of Entomology, Plant Pathology and Nematology at the University of Idaho, USA. He works on field and laboratory-based research related to biological control of invasive plants.

Philip Weyl is a weed biocontrol research scientist with CABI, Switzerland. His research focuses on developing biological control agents for invasive weeds management, including surveys, host range testing and impact assessment.