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ORIGINAL RESEARCH

Attraction of Brown Marmorated Stink Bugs, *Halyomorpha halys*, to Blooming Sunflower Semiochemicals

Warren H. L. Wong[1] · **Regine M. Gries**[1] · **Paul K. Abram**[2] · **Santosh K. Alamsetti**[1] · **Gerhard Gries**[1]

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Abstract

The polyphagous invasive brown marmorated stink bug, *Halyomorpha halys*, reportedly discriminates among phenological stages of host plants. To determine whether olfaction is involved in host plant stage discrimination, we selected (dwarf) sunflower, *Helianthus annuus*, as a model host plant species. When adult females of a still-air laboratory experiment were offered a choice of four potted sunflowers at distinct phenological stages (vegetative, pre-bloom, bloom, seeding), most females settled onto blooming plants but oviposited evenly on plants of all four stages. In moving-air two-choice olfactometer experiments, we then tested each plant stage *versus* filtered air and *versus* one another, for attraction of *H. halys* females. Blooming sunflowers performed best overall, but no one plant stage was most attractive in all experiments. Capturing and analyzing (by GC–MS) the headspace odorants of each plant stage revealed a marked increase of odorant abundance (e.g., monoterpenes) as plants transitioned from pre-bloom to bloom. Analyzing the headspace odorant blend of blooming sunflower by gas chromatographic-electroantennographic detection (GC-EAD) revealed 13 odorants that consistently elicited responses from female *H. halys* antennae. An 11-component synthetic blend of these odorants attracted *H. halys* females in laboratory olfactometer experiments. Furthermore, in field settings, the synthetic blend enhanced the attractiveness of synthetic *H. halys* pheromone as a trap lure, particularly in spring (April to mid-June). A simpler yet fully effective sunflower semiochemical blend could be developed and coupled with synthetic *H. halys* aggregation pheromones to improve monitoring efforts or could improve the efficacy of modified attract-and-kill control tactics for *H. halys*.

Keywords Plant phenology · Pentatomidae · Plant volatile compounds · Olfactometer bioassays · GC-EAD · GC–MS

Introduction

Host plants provide a plethora of benefits to insect herbivores. Plants serve as: (*i*) resources rich in nutrients (House 1961; Sinclair 2015; Urbaneja-Bernat et al. 2020), water (Kerkut and Gilbert 1985) and secondary metabolites (Boppré 1984; Honda et al. 2018); (*ii*) rendezvous sites for mate encounters (Geiselhardt et al. 2012; Xu and Turlings 2018); (*iii*) oviposition sites for gravid females (Forister 2004; Jaenike 1978; Thompson 1988); (*iv*) food sources for offspring development (García-Robledo and Horvitz 2012; Scriber and Slansky 1981; Via 1986); and (*v*) refuges from harsh weather and natural enemies (Jones and Sullivan 1981; Mulatu et al. 2004).

Locating and selecting host plants is a dynamic process that changes throughout plant and insect life cycles (Hallett 2007; Jönsson et al. 2005; Mphosi and Foster 2010). Throughout growth, maturation, and senescence, plants change in form, nutrient composition, and potential benefits that they provide to insect herbivores. For example, only in the blooming stage do herbaceous plants offer nectar and pollen for pollinators, and only plants in the fruiting stage provide resources for insect frugivores that specialize in raw fruits, nuts, and seeds.

As plants progress through phenological stages, their visual and olfactory apparency to insect herbivores changes accordingly (Mphosi and Foster 2010; Prokopy and Owens 1978). Semiochemicals (message-bearing chemicals) associated exclusively or primarily with a distinct phenological stage may guide specific insect herbivores to these plants. For example, inflorescence semiochemicals of sunflower,

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✉ Warren H. L. Wong
warren.h.l.wong@gmail.com

[1] Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada

[2] Agassiz Research and Development Centre, Agriculture and Agri-Food Canada, Agassiz, BC V0M 1A0, Canada

Helianthus annuus (Asterales: Asteraceae), attract females of the American sunflower moth, *Homoeosoma electellum* (Lepidoptera: Pyralidae), expedite maturation of their eggs, and prompt oviposition by gravid female moths on inflorescences (Arthur and Bauer 1981; McNeil and Delisle 1989). Female moths responding to these inflorescence semiochemicals essentially synchronize the development of their larval offspring with the presence of protein-rich pollen. Selecting and ovipositing on plants that maximize the survival and performance of larval offspring is particularly important for female insects whose offspring cannot readily relocate and depend on their mother's choice of the host plant (Jaenike 1978; Valladares and Lawton 1991). This "mother knows best" principle likely applies to many insect herbivores.

Plant semiochemicals that attract herbivores have mostly been studied with insects that exploit single or few host plant species. For example, crucifer-specific volatile metabolites of glucosinolates attract the cabbage seed weevil, *Ceutorhynchus assimilis* (Blight et al. 1995), and 6-methoxy-2-benzoxalinone coupled with CO_2 attracts western corn rootworm larvae, *Diabrotica virgifera*, which are oligophagous on roots of maize and some other grasses (Bjostad and Hibbard 1992). Much less is known about how polyphagous insects such as stink bugs (Hemiptera: Pentatomidae) discriminate among phenological stages of their host plants.

Stink bugs are pests of many crops, including tree fruits, nuts, vegetables, and row crops such as field corn and soybean (McPherson and McPherson 2000). As stink bugs commonly switch host plants throughout spring, summer, and fall, they can cause damage to multiple crops (Tillman et al. 2010; Todd 1989). For example, the invasive brown marmorated stink bug, *Halyomorpha halys*, appears to discriminate among both different host plant species as well as among different phenological stages of single host plant species (Bergmann et al. 2016; Blaauw et al. 2019; Morrison et al. 2016b; Nielsen et al. 2016). However, the underlying mechanisms of host plant recognition by *H. halys* are still unknown (Morrison et al. 2018). Plant semiochemicals play an important role, although in one recent study apple and peach odorants, coupled with green leaf volatiles, failed to attract *H. halys* or to enhance their attraction to the *H. halys* aggregation pheromone (Morrison et al. 2018), which has previously been identified (Khrimian et al. 2014). As *H. halys* is attracted to host plants at distinct phenological stages (Lee et al. 2013; Nielsen et al. 2016), the odor profile of these attractive stages warrants analysis.

Choosing a host plant that is highly preferred by *H. halys* is the first step towards identifying plant semiochemicals attractive to *H. halys*. Sunflower is a good candidate for semiochemical analyses because it is a candidate trap crop for *H. halys* to protect nearby cash crops (e.g., peppers) (Soergel et al. 2015). Moreover, as trap crops in one previous study, blooming sunflowers were more effective than sorghum, admiral pea, millet, and okra (Nielsen et al. 2016). Among host plants capable of attracting and retaining *H. halys*, blooming-stage sunflowers appear to be particularly attractive to *H. halys* (Blaauw et al. 2017; Nielsen et al. 2016). Conceivably, their attractiveness is due to inflorescence semiochemicals, although visual and thermal inflorescence cues cannot be discounted.

Our objectives were to: (1) determine whether *H. halys* discriminates between phenological stages of sunflower (vegetative, pre-bloom, bloom, seed) for settling and oviposition; (2) compare the relative attractiveness of each phenological stage to *H. halys*; (3) analyze the odor profile of each stage; and (4) prepare a synthetic blend of odorants present in the most attractive stage and bioassay this blend for its attractiveness to *H. halys* in laboratory and field experiments.

Material and Methods

Insect Collection and Rearing

Halyomorpha halys was reared in the insectary of the Agassiz Research and Development Centre (ARDC), British Columbia, Canada. Annually, laboratory colonies were replenished with specimens captured in the nearby city of Chilliwack. Insects were maintained at a photoperiod of 16L:8D, 40–50% relative humidity, and a temperature of 24 ± 1 °C. Eggs were placed into separate containers (12.5 × 12.5 × 15 cm, Insect Pot; BugDorm, Tai Chung, Taiwan), and early instars provisioned with organic beans and pumpkin seeds ad libitum. Later instars were transferred to mesh cages (45 × 45 × 45 cm; BugDorm), each containing two potted pepper plants and two potted fava bean plants. The bugs' diet was supplemented with pumpkin seeds ad libitum placed on the rooftop of cages. Adults were transferred to new cages (45 × 45 × 45 cm), each containing two potted pepper plants, two potted cabbage plants, a single organic carrot, and a banana; pumpkin seeds were placed on top of cages. Adult, 2- to 3- week-old, reproductively mature females were used in experiments.

Growing Dwarf Sunflowers

In a greenhouse at ARDC, 24 pots (15.5 cm × 16.5 cm) filled with bedding blend soil (Sumas Gro Media, Chilliwack, BC, CA) were each seeded with a single sunflower seed (var. Big Smile; *Helianthus annuus*; Johnny's Selected Seeds, ME, USA) once weekly. Seedlings were moistened with plain water and grown under a 16L:8D photoperiod, 10–40% RH, and day- and night-time temperatures of 25–27 °C and 23–25 °C, respectively. After the first pair of true leaves had formed (about one week after seeding), plants were watered with fertilizer-enriched water (18–6-20 fertilizer EC: 1.8–2.0 mS/cm, pH: 6.0–6.4; Terralink, Abbotsford, BC, CA) until they reached the pre-bloom stage (after 6 weeks of growth). To control for powdery mildew, all plants were treated once a week with the biofungicide Rhapsody (20 ml/L; AgraQuest Inc., CA, USA). As needed, herbivore insect pests were suppressed with commercially available biological control agents. Once every month, all pots were drenched with a formulation of a nematode biological control agent (Nemasys; Becker Underwood Inc., IA, USA; mixed at 250 million per 9.5 L of water), using the greenhouse injector system.

Phenological Stages of Sunflowers

To study the effect of sunflower phenological stage on the attraction of *H. halys*, four distinct phenological stages (Schneiter and Miller 1981) were selected: vegetative, pre-bloom, bloom, and seed (Fig. 1A). Assignments of plants to these stages were based on specific criteria, as follows: vegetative-stage plants had a foliar diameter of ≤ 5 cm and lacked a budding flower head; pre-bloom plants had a pedicel length of > 2 cm but no visible flower petals; blooming plants displayed clearly-visible yellow petals, whereas plants at the seeding stage had already desiccated or absent flower petals and developed seed.

Settling and Oviposition by *H. halys* on Sunflower in Four Phenological Stages

To determine the phenological stage(s) of sunflower that *H. halys* females select for feeding and oviposition, one potted plant of each stage (vegetative, pre-bloom, bloom, seed) was placed into a cage (60 × 60 × 120 cm; BioQuip Products Inc., CA, USA) at a randomly assigned position (Fig. 1A; Exp. 1). Then, 15 females were released at the center of the cage and their position was recorded 24 h and 48 h later, counting only females in physical contact with a plant surface. Recording the position of females at two intervals enabled us to gauge the consistency of the females' plant choice over time. After 48 h, all females were returned to the lab colony not to be used in other experiments, and each plant was destructively sampled to count the number of eggs it had received. The experiment was replicated 20 times.

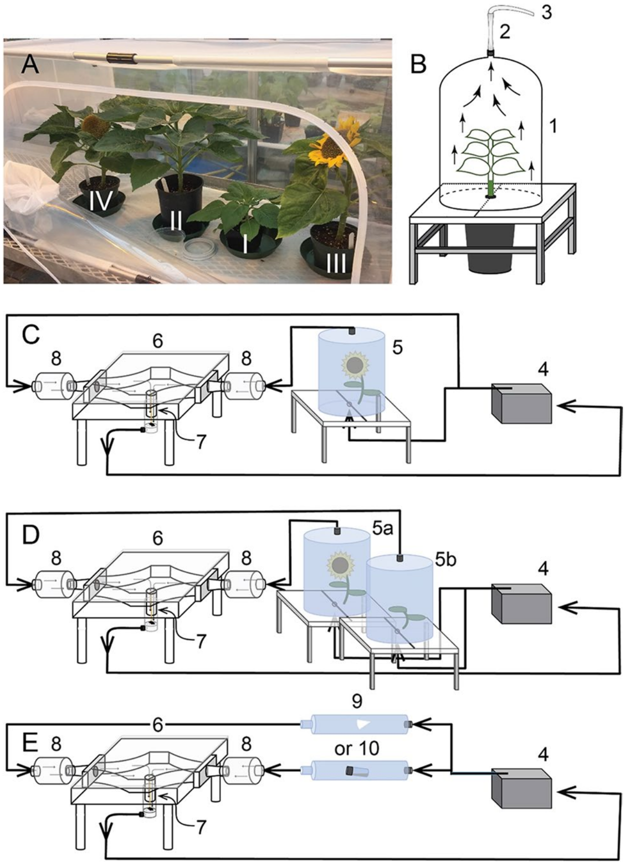

Fig. 1 Illustrations of greenhouse and laboratory experimental designs (drawings not to scale). **A**, Still-air, four-choice design offering distinct phenological stages of potted sunflower plants (I, vegetative; II, pre-bloom; III, bloom; IV, seed) for feeding and oviposition responses by female brown marmorated stink bugs. **B**, Housing for potted sunflower plants consisting of a 'guillotine' adjustable-height stand with a tall dome enclosing the plant (1), an odorant trap (2), and Teflon tubing (3) connecting the housing to the olfactometer system. **C-E**, Olfactometer system comprising an air filtration/purification station (4), various odor sources as test stimuli [one or two potted live plants (5, 5a, 5b); a synthetic odorant blend emanating either from filter paper (9) or a mineral oil formulation (10)], the olfactometer arena (6), an insect release chamber (7), and an insect trap (8) retaining stink bugs that made a decision

Acquisition of Sunflower Headspace Odorants

To determine whether the four phenological stages of sunflower (vegetative, pre-bloom, bloom, seed) differ in their headspace odorants, three potted plants of each stage were transferred from the ARDC greenhouse facility to the Gries-lab at Simon Fraser University (SFU). Each plant was placed into a "guillotine" adjustable-height stand (13.6 × 13.6 × 2.5 cm; Sigma Scientific LLC, FL, USA; Fig. 1B1), with the entire (intact) plant enclosed within a borosilicate glass dome (31.8 cm diameter × 45.7 cm), and the plant stem residing in the central opening (2.5 cm diameter) formed by two abutting platforms. Void space between the plant stem and the platforms was covered with a strip of activated carbon to purify the air before entering the dome. A vacuum pump (GAST miniature diaphragm, 15D 1150 series; IDEX Corp., MI, USA) drew carbon-filtered air at 1 L min^{-1} for 24 h through the glass dome and an odorant trap connected to an outlet at the top of the dome. The odorant trap consisted of a glass tubing (10 × 0.7 cm) containing the adsorbent Porapak Q (50 mg; 100–120 mesh; Supleco, MI, USA; Fig. 1B2) and was connected via teflon tubing (0.6 cm diameter; Sigma Scientific; Fig. 1B3) to the vacuum pump. Plant-derived odorants were adsorbed on Porapak Q and desorbed with consecutive rinses of HPLC-grade pentane (2 ml) and ether (2 ml) stored in separate vials for analyses.

GC–MS and GC-EAD Analyses of Sunflower Headspace Odorants

After adding dodecyl acetate as an internal standard, each extract was concentrated to 250 µl. Aliquots (2 µl) of extracts were analyzed by coupled gas chromatography—mass spectrometry (GC–MS), using a Varian Saturn Ion Trap GC–MS (Agilent Technologies Inc., Santa Clara, CA, USA) operated in full-scan electron impact mode and fitted with a DB-5 MS column (30 m × 0.25 mm I.D., film thickness: 0.25 µm; Agilent J&W GC), with helium as the carrier gas (35 $cm^{-1\ s}$). The injector port was set at 250 °C, the transfer line at 280° C, and the ion trap at 200 °C. For analyses, the following temperature program was run: 50 °C for 5 min, 10 °C · min^{-1} to 280 °C (held for 10 min). Odorants were identified by comparing their retention indices (relative to straight-chain alkanes) (Van Den Dool and Kratz 1963) and their mass spectra with those of authentic standards purchased from Sigma-Aldrich (St. Louis, MO, USA) or synthesized in the Gries-lab (*E*3,*E*7-4,7-dimethyl-1,3,7-nonatriene; *E*3,*E*7-4,8,12-trimethyl-trideca-1,3,7,11-tridecatetraene) following previously reported protocols (Maurer et al. 1986). All compounds were > 95% chemically pure except for ocimene (90%) which also consisted of a mixture of *cis*- and *trans*-isomers (25/75).

The absolute configuration of chiral odorants was determined on a Cyclodex B column (30 m × 0.25 mm I.D., film thickness: 0.25 µm; J&W Scientific, Folsom, CA, USA) in a 5890 GC. Using a split injection mode (10:1 split ratio), α-pinene, camphene, sabinene, β-pinene, and limonene were each chromatographed isothermally at 80 °C, and borneol and bornyl acetate at 110 °C.

Aliquots of extracts were further analyzed by gas chromatographic-electroantennographic detection (GC-EAD), with procedures previously detailed (Arn et al. 1975; Gries et al. 2002). Briefly, the GC-EAD system consisted of a Hewlett-Packard 5890 GC fitted with a DB-5 GC column (30 m × 0.32 mm I.D., film thickness: 0.25 µm; Agilent J&W column, Agilent Technologies Inc.), with helium as the carrier gas (35 cm · s^{-1}). The injector port and flame ionization detector (FID) were set at 260 °C, and the following oven program was used: 50 °C for 1 min, then 20 °C · min^{-1} to 280 °C. For GC-EAD recordings, an antenna was carefully dislodged from a female's head and suspended between two glass capillary electrodes (1.0 × 0.58 × 100 mm; A-M Systems, Carlsborg, WA, USA) prepared to accommodate the antenna, and filled with a saline solution (Staddon and Everton 1980).

General Design of Laboratory Olfactometer Experiments

The attraction of *H. halys* females to test stimuli was tested in laboratory olfactometer experiments, with visual cues being absent or occluded. The 4–choice arena olfactometer (Sigma Scientific LLC; Fig. 1C, D, E) was modified for 2-choice experiments and was illuminated from above with an F32T5 daylight bulb and an F32T8 plant and aquarium fluorescent light bulb (Philips, Amsterdam, NL). To deliver test stimuli originating from odor sources (potted sunflower, headspace odorant extract of sunflower, synthetic odor blend), we used pressurized and purified air delivered through polyethylene and Teflon tubing (6.35 mm diameter). Filter-purified air (Fig. 1C4) moved at 1 L · min^{-1} through the olfactometer system entering – in sequence – the stimulus chamber(s) [a glass dome (31.8 cm diameter × 45.7 cm; Fig. 1C5, D5) or an inline glass chamber (1.8 cm diameter × 7.5 cm; Fig. 1E9, E10)], a barrel-shaped insect trap (6.5 cm diameter × 12.7 cm; Fig. 1C8, D8, E8), the choice arena (30.5 cm × 30.5 cm × 2.5 cm; Fig. 1C6, D6, E6), and the insect release chamber (2.4 cm diameter × 7.5 cm; Fig. 1C7, D7, E7), before returning to the air filter system (Fig. 1C4). For each replicate, a single *H. halys* female was placed into the release chamber fitted with a wooden stick and allowed to enter the choice arena on her own accord and to select a test stimulus. A choice was recorded when the female had entered one of the two insect traps within 45 min (in experiments testing a treatment stimulus *vs* a filtered air or mineral oil control) or within 60 min (in experiments testing two live plants). Extending the upper time limit to 60 min in choice experiments with two plants took into account that females faced difficult choices between two complex test stimuli. Following each replicate, both the arena and the traps were cleaned with Sparkleen (Fisher Scientific Comp., ON, CA) and the wooden climbing stick was replaced.

Specific Laboratory Olfactometer Experiments

Experiments 2–5 (n = 30 each) tested attraction of *H. halys* females to potted sunflower at each of four phenological stages: vegetative (Exp. 2), pre-bloom (Exp. 3), bloom (Exp. 4), and seed (Exp. 5), all *vs* filtered air. Follow-up experiments 6–11 (n = 30 each) used a full factorial design, testing the attractiveness of potted plants at each phenological stage *vs* that of each of the other three plant stages. Specifically, the vegetative stage was tested *vs* the pre-bloom, bloom, and seed stage, respectively (Exps. 6–8). Moreover, the pre-bloom stage was tested *vs* the bloom and seed stage, respectively (Exps. 9–10), and the seed stage was tested *vs* the bloom stage (Exp. 11).

Drawing on combined data of behavioral experiments 1–11 (see Results), it appeared that the bloom stage was most effective overall in prompting settling and attraction responses by female *H. halys*. Moreover, there was a strong shift in the abundance and occurrence of odorants as plants transitioned from pre-bloom to bloom. Therefore, a synthetic blend (SB) of odorants was prepared that resembled the amount and ratio of natural odorants released from blooming plants during 24 h. Only those odorants (boldfaced in Table 1) that elicited antennal responses (see Results) were included in the SB. This SB was then tested for its attractiveness to *H. halys* using various delivery systems. First, SB (0.1 ml) was applied onto filter paper and tested *vs* a filtered air control (Exp. 12).

To help decide whether mineral oil (MO) was suitable as a dissemination medium for SB in field experiments, we needed to determine whether MO was behaviorally benign to *H. halys* and thus tested MO *vs* filtered air (Exp. 13). With evidence that MO was not attractive to *H. halys* (see Results), we proceeded to test SB formulated in MO at a low dose (0.01 ml SB in 0.5 ml MO) and a higher dose (0.1 ml SB in 0.5 ml MO) *vs* plain MO (Exps. 14, 15). The 24-h odorant release rate of the high-dose formulation on days 1, 6, 9, 10, 12, and 13 (Table 1) was equivalent to

Table 1 Composition and amounts of odorants in headspace odorant extract of potted sunflower at four distinct phenological stages. Odorants were collected from four plants per stage for 24 h each

Plant odorant	Phenological stage of potted sunflower			
	Vegetative	Pre-blooming	Blooming	Seeding
	Mean[1] (SEM)	Mean (SEM)	Mean[2,3] (SEM)	Mean (SEM)
*Z*3-hexenol	5.2 (0.8)	3.3 (1.4)	1.0 (0.7)	1.3 (0.5)
xylene isomer 1	18.3 (13.2)	7.2 (4.4)	0.4 (0.1)	3.6 (2.7)
xylene isomer 2	81.0 (62.7)	30.1 (19.7)	2.6 (1.1)	18.0 (13.0)
xylene isomer 3	31.4 (18.7)	13.9 (4.1)	4.7 (1.7)	8.0 (3.7)
thujene	4.7 (1.1)	1.5 (0.6)	0.7 (0.1)	4.7 (1.9)
(±)-α-pinene [(–)-isomer = 90%]	88.1 (21.2)	221.5 (88.6)	**655.7 (221.5)**	**1467.4 (370.6)**
(–)-camphene	13.2 (3.2)	8.7 (2.3)	**36.6 (5.1)**	**47.1 (18.3)**
(–)-sabinene	63.3 (15.6)	44.8 (6.1)	**108.8 (18.8)**	**298.1 (87.3)**
(±)-ß-pinene [(–)-isomer = 60%]	14.9 (4.2)	10.3 (2.0)	**49.2 (16.4)**	**106.5 (27.0)**
sulcatone	7.8 (1.9)	3.4 (0.9)	8.1 (3.2)	13.2 (2.9)
unknown hydrocarbon	9.3 (2.9)	4.8 (1.9)	1.0 (0.2)	2.9 (1.1)
(+)-limonene	11.6 (2.3)	9.6 (2.1)	7.9 (0.9)	20.5 (5.8)
cis/trans-ocimene (30/70)	6.3 (1.5)	1.8 (0.6)	0.7 (0.3)	0.8 (0.2)
nonanal	8.5 (1.3)	3.9 (1.5)	1.5 (0.2)	2.2 (0.8)
_E_3,_E_7-4,7-dimethyl-1,3,7-nonatriene	20.2 (9.6)	10.4 (1.7)	21.3 (3.1)	7.3 (2.1)
verbenol	0 (0)	0 (0)	1.7 (0.4)	3.0 (1.7)
(–)-borneol	0 (0)	0 (0)	**2.7 (0.3)**	**4.7 (2.5)**
unknown hydrocarbon	54.4 (16.8)	13.3 (0.9)	4.4 (1.2)	7.2 (1.9)
decanal	9.5 (2.1)	2.5 (0.1)	0.8 (0.9)	1.6 (8.9)
(–)-bornyl acetate	0 (0)	0.4 (0.9)	**10.9 (0.1)**	**27.9 (0.6)**
unknown unsaturated hydrocarbon	59.4 (18.3)	15.1 (2.1)	4.4 (1.6)	27.5 (13.5)
unknown sesquiterpene	6.4 (3.8)	7.8 (1.5)	8.5 (2.2)	3.5 (1.0)
unknown hydrocarbon	6.1 (1.7)	1.4 (0.3)	0.6 (0.3)	1.0 (0.4)
trans-caryophyllene	10.1 (1.6)	6.9 (1.6)	5.6 (1.2)	4.0 (1.6)
unknown sesquiterpene	15.1 (4.3)	4.4 (1.9)	7.1 (2.4)	2.3 (0.51)
_E_3,_E_7-4,8,12-trimethyl-1,3,7,11-tridecatetraene	4.0 (15.8)	2.9 (3.6)	**9.5 (1.1)**	**1.9 (1.9)**

Bold-faced compounds were included in a synthetic blend for laboratory and field testing

[1]Mean = amount (ng) of compound per 1 g of fresh weight of plant; [2]Mean fresh weight of four plants: 167.75 g; [2]mean amount of all compounds (or all bold-faced compounds) released from intact plants during 24 h: 160.4 µg (146.5 µg); [3]the mean amounts of all bold-faced compounds released from a synthetic blend (0.1 ml formulated in 0.5 ml of mineral oil) on days 1, 6, 9, 10, 12, and 13 were 7,000 µg, 10,050 µg, 5,275 µg, 5,375 µg, 2,275 µg, and 2,850 µg, respectively (note: compound ratios remained comparable with those reported in this table)

that of 47, 68, 36, 36, 15, and 19 blooming sunflowers, respectively.

Finally, to determine whether the time of season affects the propensity of *H. halys* to respond to the SB, we tested the attraction of *H. halys* exposed to a long photophase (16L:8D; laboratory colony) or a shorter photophase (11L:13D; field-collected specimens) (Exps. 16, 17).

Field Testing the Synthetic Odorant blend (SB)

The ability of the synthetic odorant blend (SB) to attract *H. halys*, or to enhance the attractiveness of a commercial *H. halys* pheromone lure [(3*S*,6*S*,7*R*,10*S*)- and (3*R*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolene-3-ol (Khrimian et al. 2014) *plus* synergistic methyl (2*E*,4*E*,6*Z*)-decatrienoate (Leskey et al. 2015) (Stink bug DUAL Lures; Trécé, Inc., OK, USA)], was tested in a field experiment in BC. The experiment was run from 26 August to 22 October 2019 when *H. halys* adults move from host plants to overwintering sites, and from 16 March to 03 July 2020 when *H. halys* adults emerge from overwintering diapause, and then aggregate on host plants to feed, mate, and then lay eggs. In each of three field sites (Rosedale, 49.170965, -121.830512; Chilliwack, 49.158057, -121.998131; Abbotsford, 49.003225, -122.264077), three replicates with six treatments each were set up, with > 10 m spacing between replicates. In each replicate, an adhesive-coated trap (15.2 × 30.5 cm; Trécé, Inc., OK, USA) was affixed 1.2 m above ground to one of six wooden stakes which were spaced > 5 m apart. This spacing was selected to account for pheromone plume reach (< 3 m) of pheromone-baited sticky traps (Kirkpatrick et al. 2019). Each trap was randomly assigned to one of six treatments: (1) commercial pheromone lure ('P'); (2) pheromone lure *plus* mineral oil ('P + MO'); (3) a synthetic blend of sunflower odorants in mineral oil ('SB in MO') (see above); (4) 'SB in MO + P'; (5) MO; and (6) an unbaited control. MO (0.5 ml), and SB (0.1 ml) formulated in 0.5 ml of MO, were each presented in a dram vial (1.5 × 4.5 cm; Fisher Scientific Comp. ON, CA) affixed to wooden stakes above the adhesive trap. As SB lures had consistent release rates for up to 14 days, lure replacement at only bi-weekly intervals was justified. All lures were rain-sheltered by a horizontal clear plastic cover (2.5 × 5 cm). Once each week for the entire duration of the field experiment, trap captures of *H. halys* were recorded, and treatment positions within replicates were re-randomized. Traps with > 10 *H. halys* captured were replaced, and all other *H. halys* were dislodged from the adhesive surface of traps.

Statistical Analysis

Data of laboratory experiment 1, which tested feeding and oviposition choices of *H. halys* on sunflower in the vegetative, pre-bloom, bloom, and seed stage, were analyzed with an exact multinomial test, comparing the bugs' choices against a theoretical choice distribution of 25:25:25:25%. This analysis was followed by a Fisher's exact test to correct for multiple comparisons with small sample sizes. All two-choice olfactometer data (Exps. 2–17) were analyzed with a χ^2 test under the null hypothesis that *H. halys* have no preference for either of the two stimuli tested.

Trap catch data of adult *H. halys* in the 6-treatment field experiment (Exp. 18) were analyzed with a zero-inflated generalized linear mixed model (GLMM) with a negative binomial error distribution, using treatment as a categorical fixed factor, and site and week as categorical random factors. Likelihood ratio tests were used to assess statistical significance ($p < 0.05$). For post-hoc multiple comparisons among categorical treatments, Tukey multiple comparisons tests were run. Trap catch data of nymphs in the fall of 2019 are reported in supplementary materials. Total trap captures of nymphs in the spring/summer of 2020 were insufficient to warrant statistical analyses (see 'Results' for total nymph captures).

All statistics were run with R version 3.6.2 (R Core Team 2020) using RStudio version 1.2.5033 (RStudio Team 2016). Software packages used for analyses included 'EMT' (Menzel and Menzel 2015) for the multinomial analysis, 'car' (Fox et al. 2012) for likelihood ratio tests, and 'multcomp' (Hothorn et al. 2013), 'multcompView' (Graves et al. 2015) and 'emmeans' (Lenth et al. 2018) for Tukey multiple comparisons and extraction of model estimates and confidence intervals (Fig. 2).

Results

Settling and Oviposition of *H. halys* on Sunflower in Four Phenological Stages

Of the total of 300 adult females tested in 20 replicates of experiment 1, 154 (51.3%) and 193 females (64.3%) were present on plants 24 h and 48 h, respectively, after experiment initiation. These females discriminated among the four phenological stages of potted sunflower plants that were offered (24 h: two-sided Fisher's Exact Test, $p = 0.002$; 48 h: $p = 0.0005$) (Fig. 3). Most females selected plants at the bloom stage (24 h: 70/154; 48 h: 86/193), whereas fewer females selected plants at the seed stage (24 h: 43/154; 48 h: 53/193), pre-bloom stage (24 h: 34/154; 48 h: 51/193), and vegetative stage (24 h: 7/154; 48 h: 3/193).

When plants were destructively sampled for the presence of egg masses 48 h after the experiment was initiated, the number of egg masses on each plant was independent of the phenological stage (two-sided Fisher's Exact Test, $p = 0.1084$). Numerically, plants in the seed stage received

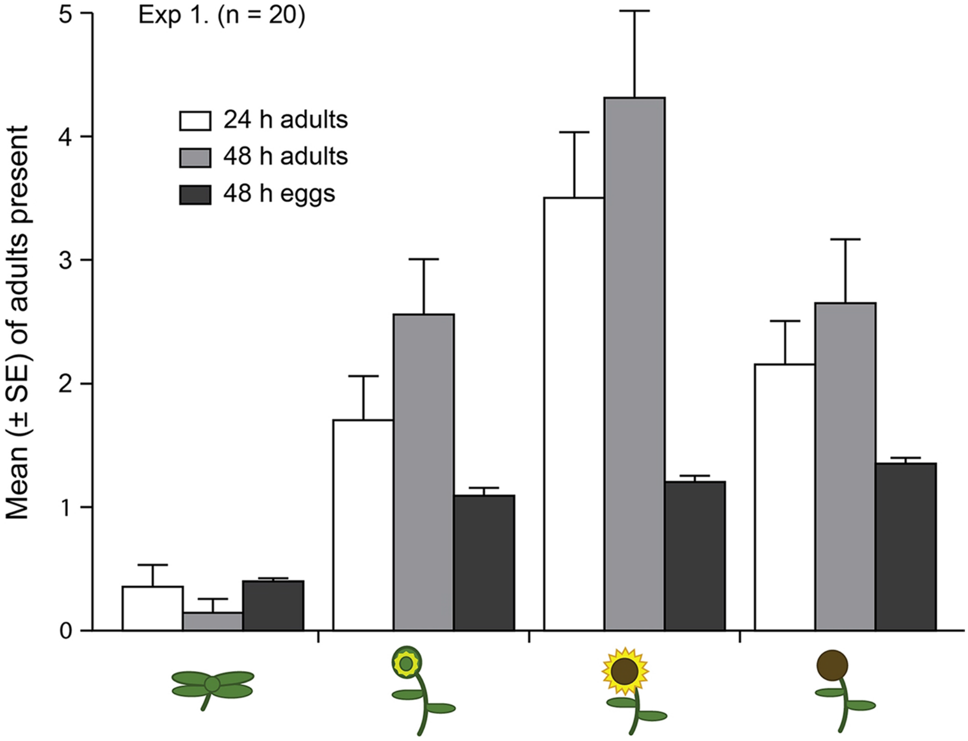

Fig. 2 Settling and oviposition by female brown marmorated stink bugs in response to concurrently offered potted sunflower plants at four phenological stages of development (from left to right: vegetative, pre-bloom, bloom, seed) (see Fig. 1A). Settling responses of females depended upon plant phenological stage, as recorded 24 h and 48 h after experiment initiation (24 h: two-sided Fisher's Exact Test, $p = 0.002$; 48 h: $p = 0.0005$). In contrast, plant phenological stage had no significant effect on numbers of egg masses deposited (two-sided Fisher's Exact Test, $p = 0.10$)

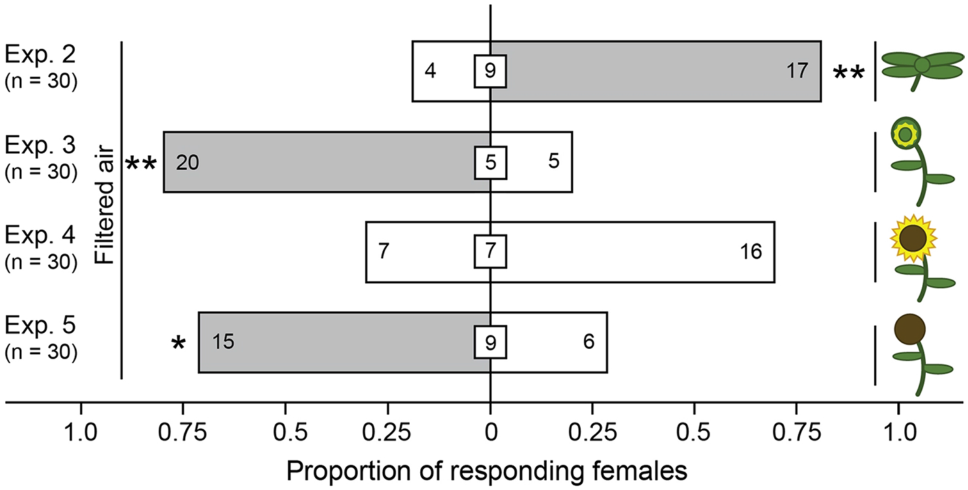

Fig. 3 Responses of adult female brown marmorated stink bugs in moving-air olfactometer experiments 2–5 (Fig. 1C) when offered a choice between filtered air as the control stimulus and a potted sunflower plant as the treatment stimulus (for plant symbols see Fig. 3). Shown in bars and insert boxes are the number of females selecting a treatment or control stimulus and not responding to test stimuli, respectively. For each experiment, an asterisk indicates a significant preference for a test stimulus (χ^2 test; * $p \leq 0.05$, ** $p \leq 0.01$)

more egg masses (27/81) than blooming plants (24/81), pre-bloom plants (22/81), or plants in the vegetative stage (8/81).

Olfactory Attraction of *H. halys* to Potted Sunflowers

In olfactometer experiments 2–5 ($n = 30$ each; Fig. 3) with filtered air as the control stimulus and a potted sunflower plant as the treatment stimulus, the phenological stage of plants affected the females' responses. Vegetative-stage plants attracted females (Exp. 2: 17 *vs* 4, $\chi^2 = 8.05$, $p = 0.0046$), pre-bloom plants deterred females (Exp. 3: 5 *vs* 20, $\chi^2 = 9.00$, $p = 0.0027$), blooming plants (not significantly) attracted females (Exp. 4: 16 *vs* 7, $\chi^2 = 3.52$, $p = 0.06$), and seed-stage plants deterred females (Exp. 5: 6 *vs* 15, $\chi^2 = 3.86$, $p = 0.049$).

Comparing the attractiveness of plants head-to-head in a full factorial design (Fig. 4), pre-bloom plants (Exp. 6), but neither blooming plants (Exp. 7) nor seeding plants (Exp. 8), attracted more females than vegetative-stage plants (Exp. 6: 18 *vs* 6, $\chi^2 = 6.00$, $p = 0.0014$; Exp. 7: 7 *vs* 12, $\chi^2 = 1.32$, $p = 0.25$; Exp. 8: 16 *vs* 11, $\chi^2 = 0.93$, $p = 0.33$). Compared to pre-bloom plants, neither blooming plants (Exp. 9) nor seeding plants (Exp.10) were more attractive to females (Exp. 9: 13 *vs* 11, $\chi^2 = 0.17$, $p = 0.68$; Exp. 10: 8 *vs* 18, $\chi^2 = 3.85$,

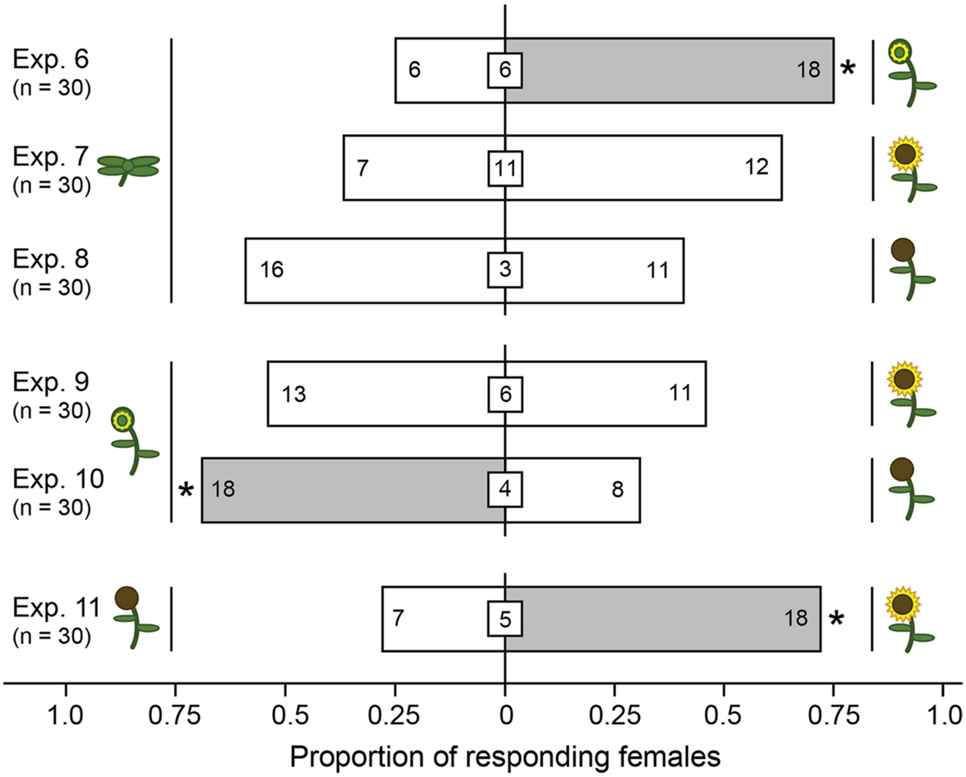

Fig. 4 Responses of adult female brown marmorated stink bugs in moving-air olfactometer experiments 6–11 (Fig. 1D) when offered a choice between two potted sunflower plants at different stages of phenological development (for plant symbols see Fig. 3). Shown in bars and insert boxes are the number of females selecting a plant and not responding to plants, respectively. For each experiment, an asterisk (*) indicates a significant preference for a test stimulus (χ^2 test, $p \leq 0.05$)

$p = 0.045$). However, blooming plants attracted more females than seeding plants (Exp. 11: 18 *vs* 7, $\chi^2 = 4.84$, $p = 0.027$).

GC–MS and GC-EAD Analyses of Sunflower Headspace Odorants

GC-EAD analyses of Porapak Q headspace odorant extract of blooming sunflowers revealed five monoterpene hydrocarbons (α-pinene; camphene; sabinene; β-pinene; limonene), one sesquiterpene hydrocarbon (*trans*-caryophyllene), two additional hydrocarbons (*E*3,*E*7-4,7-dimethyl-1,3,7-nonatriene; *E*3,*E*7-4,8,12-trimethyl-1,3,7,11-tridecatetraene), one alcohol (borneol), one acetate (bornyl acetate), one ether (2-methylanisole) and one aldehyde (nonanal) that consistently elicited responses from female *H. halys* antennae (Fig. 5). Analyzing odorants on a GC-column that separated optical isomers of EAD-active odorants further revealed that both α- and β-pinene occurred as a mixture of (+)- and (–)-isomers [α-pinene; (+)(10%)/(–)(90%); β-pinene: (+)(40%)/(–)(60%)], whereas (–)-camphene, (+)-limonene, (–)-borneol, and (–)-bornyl acetate were each present as a single optical isomer. While most odorants were present at each of the four phenological stages of sunflower, the relative abundance of these odorants changed in relation to the plant stage. Particularly, α- and β-pinene, camphene, and sabinene markedly increased as plants transitioned from pre-bloom to bloom (Table 1). Borneol and bornyl acetate were absent at the vegetative stage and appeared at appreciable amounts only in the bloom and seed stages. Of the odorants with elevated abundance at the blooming stage, only *E*3,*E*7-4,7-dimethyl-1,3,7-nonatriene, and *E*3,*E*7-4,8,12-trimethyl-1,3,7,11-tridecatetraene decreased again at the seeding stage (Table 1).

Olfactory Attraction of *H. halys* to Synthetic Sunflower Odorants

The synthetic blend (SB) of blooming sunflower odorants (Table 1) attracted more stink bug females than a filtered-air control stimulus (Fig. 6, Exp. 12: 20 *vs* 4, $\chi^2 = 10.67$, $p = 0.0011$). In contrast, filtered-air and plain mineral oil (MO) as test stimuli were equally unattractive to females (Exp. 13: 9 *vs* 7, $\chi^2 = 0.25$, $p = 0.6171$), evidenced also by a large number of non-responding stink bugs. The SB formulated in MO at a low dose (Exp. 14), and at a higher dose (Exp. 15), attracted more stink bug females than MO alone but the effect was statistically significant only for the higher dose (Exp. 14: 16 *vs* 8, $\chi^2 = 2.67$, $p = 0.1025$; Exp. 15: 19 *vs* 5, $\chi^2 = 8.17$, $p = 0.0043$).

The numbers of field-captured adult *H. halys* differed among the six experimental treatments tested both in the fall (26 August to 22 October) of 2019 (Type II Wald test: $\chi^2 = 137.83$; df = 5, 476; $p < 0.0001$), and in the spring/summer (16 March to 03 August) of 2020 (Type II Wald test: $\chi^2 = 106.4$; df = 5, 1070; $p < 0.0001$) (Fig. 7, Exp. 18). While the synthetic odorant blend of blooming sunflower

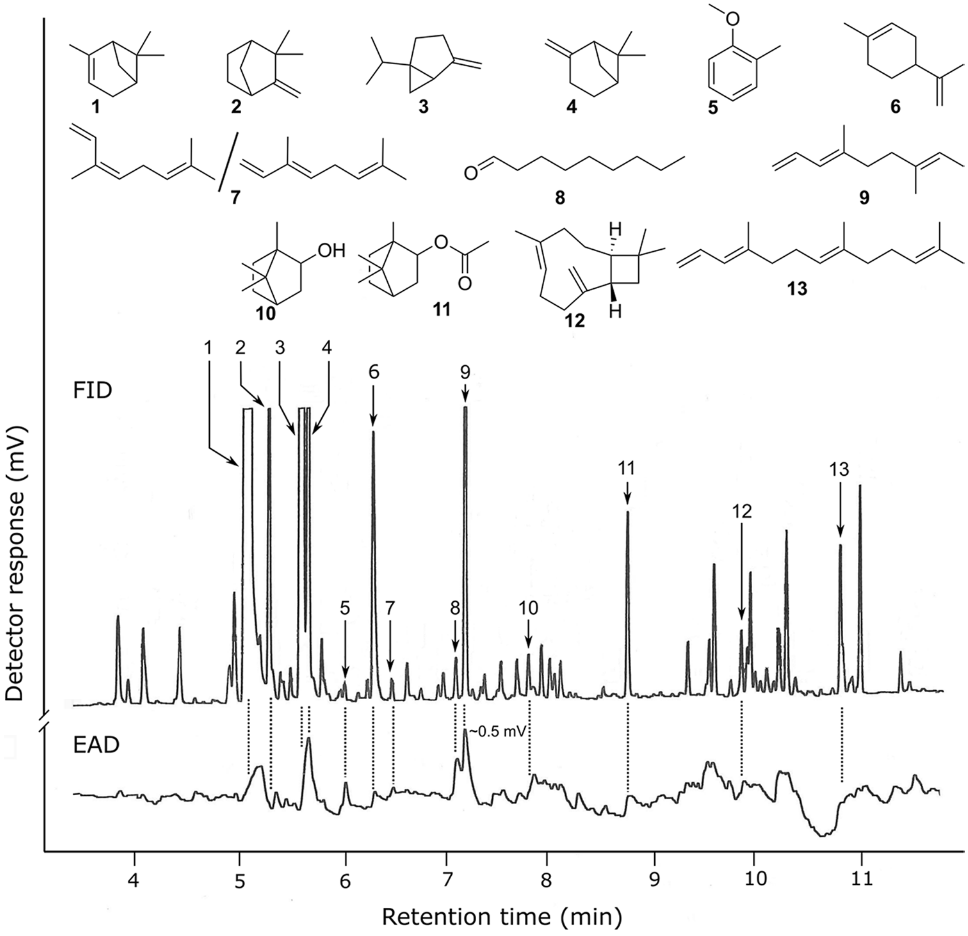

Fig. 5 Representative responses of a gas chromatographic flame ionization detector (FID) and an electroantennographic detector (EAD: antenna of a female brown marmorated stink bug) to aliquots of Porapak Q headspace odorant extract from a potted blooming sunflower. The odorants that consistently elicited antennal responses were α-pinene [(+)/(–): 10/90)] (1), (–)-camphene (2), (–)-sabinene (3), β-pinene [(+)/(–): 40/60)] (4), 2-methyl-anisole (5), (+)-limonene (6), *cis/trans*-ocimene (25/75) (7), nonanal (8), *E*3,*E*7-4,7-dimethyl-1,3,7-nonatriene (9), (–)-borneol (10), (–)-bornyl acetate (11), *trans*-caryophyllene (12), and *E*3,*E*7-4,8,12-trimethyl-trideca-1,3,7,11-tridecatetraene (13)

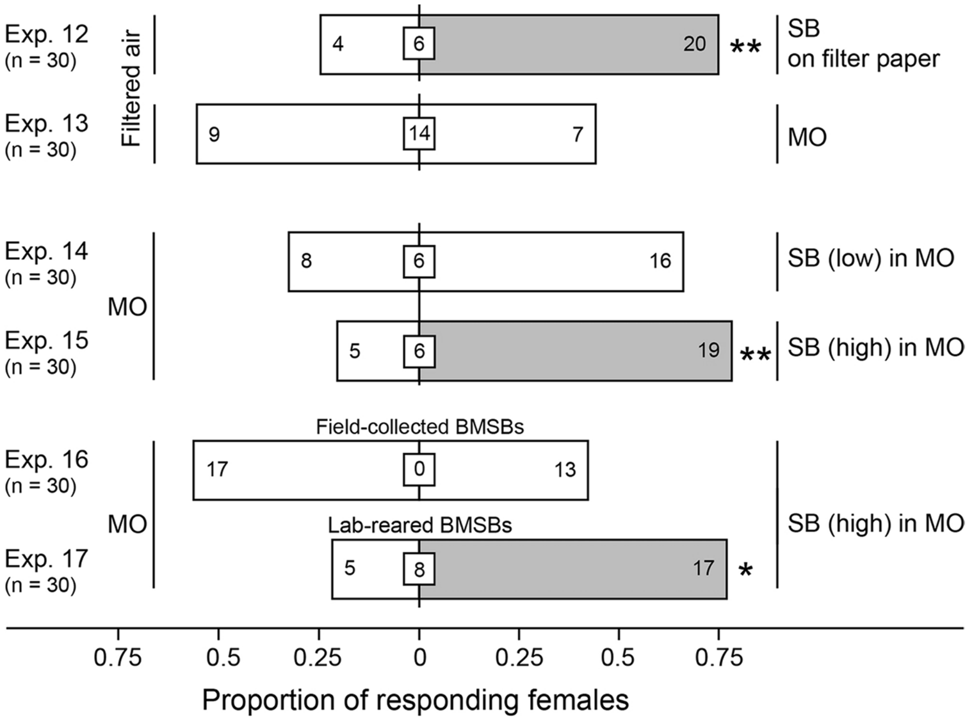

Fig. 6 Responses of adult female brown marmorated stink bugs in moving-air olfactometer experiments 12–17 (Fig. 1E) when offered a choice between a control stimulus [filtered air or plain mineral oil (MO)] and a test stimulus [synthetic blend (SB) of blooming-sunflower odorants (Table 1) applied on filter paper or formulated in MO]. Unlike females which were field-collected in the fall (6–9 October), lab-reared females were exposed to a long photoperiod (16L:8D). Shown in bars and insert boxes are the number of females selecting a treatment or control stimulus and not responding to test stimuli, respectively. For each experiment, an asterisk indicates a significant preference for a test stimulus (χ^2 test; * $p \le 0.05$, ** $p \le 0.01$)

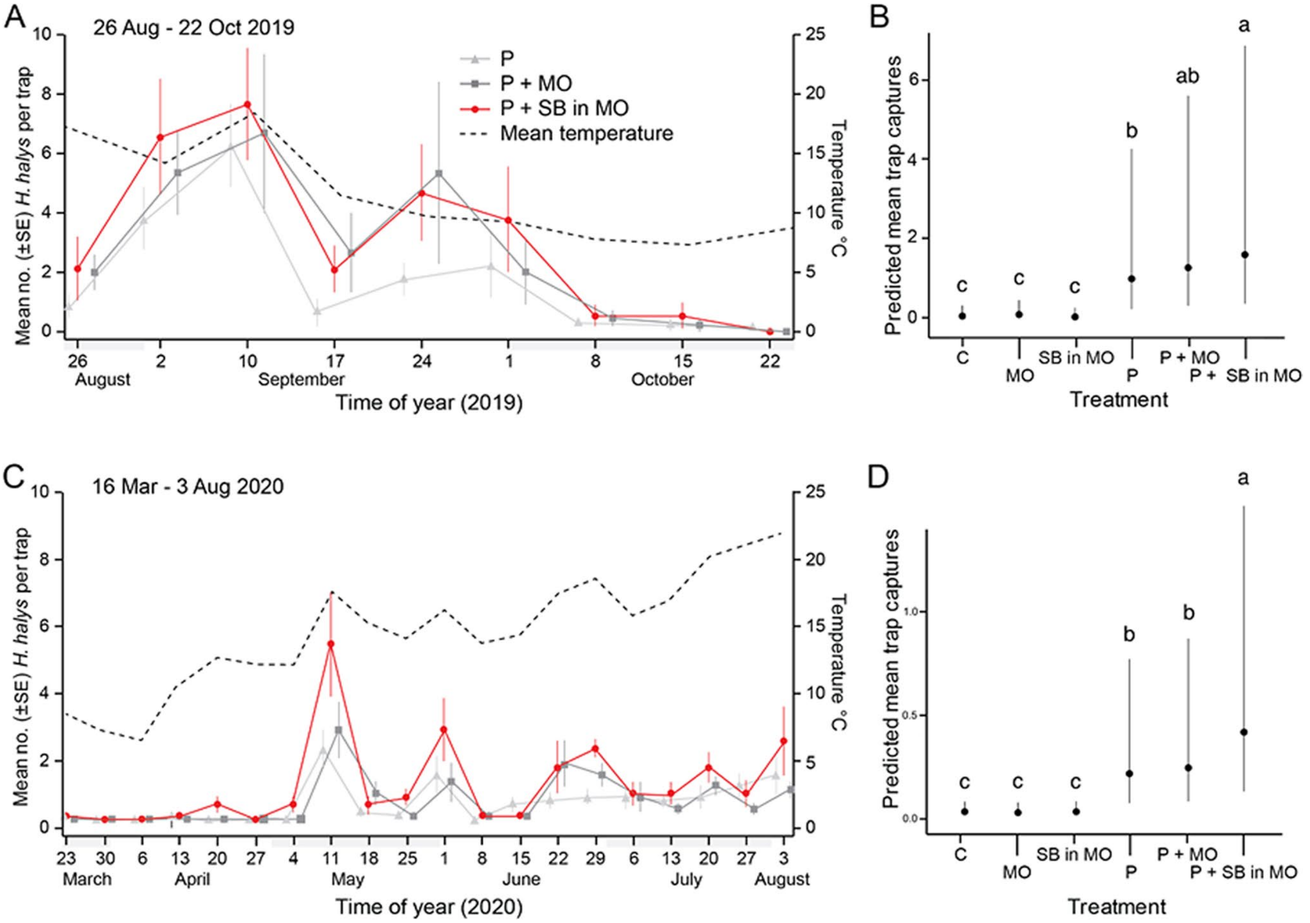

Fig. 7 Trap captures of adult brown marmorated stink bugs in a field experiment in British Columbia over time (fall 2019: panel A, spring 2020: panel **C**) and overall (fall 2019: panel B, spring 2020: panel **D**). In each of three replicates at each of three sites, traps were assigned one of six treatments: (1) an unbaited control (C), (2) mineral oil (MO), (3) a synthetic blend of sunflower odorants formulated in mineral oil (SB in MO), (4) a commercial pheromone lure (P), (5) a commercial pheromone lure plus mineral oil (P + MO), and (6) P + MO & SB in MO. Numbers of captured adult stink bugs (shown in panels **A** and **C** as among-site means for each date ± SE; shown in panels **B** and **D** as model predictions from the fitted GLMM ± 95% CI) differed among the six treatments both in the fall of 2019 (Type II Wald test: $\chi^2 = 137.83$, df = 5, $p < 0.0001$) and the spring/summer of 2020 ($\chi^2 = 106.4$, df = 5, $p < 0.0001$). In 2019 and 2020, traps baited with P + MO & SB in MO captured 62% and 104% more adult stink bugs, respectively, than traps baited with P alone (post hoc Tukey test; 2019: $p = 0.0193$; 2020: $p = 0.0001$). In panels **B** and **D**, means labelled with different letters are statistically different from each other)

(SB) formulated in mineral oil (MO) was not effective by itself, 'SB in MO' and synthetic pheromone ('P') in binary combination as a trap lure had a synergistic effect on captures of *H. halys* (see below).

In late summer and fall of 2019, traps baited with 'SB in MO + P' in binary combination captured 62% more adult *H. halys* than traps baited with 'P' alone (Tukey multiple comparison: $p = 0.019$). Pheromone-baited traps captured more adult *H. halys* than traps baited with 'SB in MO' ($p < 0.0001$), fitted merely with a 'MO' dispenser ($p < 0.0001$), or left as unbaited controls ($p < 0.0001$). Pairwise comparisons of trap captures revealed no statistical differences between each of two pairs: 'P *vs* P + MO' ($p = 0.50$) and 'P + MO *vs* P + SB in MO' ($p = 0.69$). In 2019, 450 *H. halys* nymphs were also captured (see Table S1 in Supplementary Information).

In the spring and summer of 2020, traps baited with 'SB in MO' and 'P' in binary combination captured 104% more adult *H. halys* than traps baited with 'P' alone (Tukey multiple comparison: $p = 0.0001$) and 79% more adult *H. halys* than traps baited with 'P + MO' ($p = 0.0016$). Pheromone-baited traps captured more adult *H. halys* than traps baited with 'SB in MO' ($p < 0.0001$), fitted with a MO dispenser ($p = 0.0004$), or left as unbaited controls ($p < 0.0001$); captures between traps baited with 'P' or 'P + MO' did not differ ($p = 0.97$). In 2020, 33 *H. halys* nymphs were also captured.

In both years of the field experiment, *H. halys* trap captures appear to have peaked during weeks with comparatively high ambient temperatures (Fig. 7).

Discussion

Our data support the following conclusions: (*i*) *H. halys* discriminates between phenological stages of sunflower host plants (vegetative, pre-bloom, bloom, seeding); (*ii*) headspace odorants of these plant stages differ in amount and ratio; and (*iii*) the odor blend of blooming plants – presented in synthetic form – attracts *H. halys* in laboratory bioassays, and in field settings enhances the attractiveness of synthetic *H. halys* pheromone. Below, we elaborate on these conclusions.

Population densities of *H. halys* appear to be linked to the presence of host plants at specific phenological stages (Blaauw et al. 2019; Soergel et al. 2015). Large populations are found on seeding plants (Nielsen et al. 2016), implying that they are particularly appealing to *H. halys*. In field settings, however, where most host preference studies with *H. halys* were run, seeding plants often co-occur with blooming plants (Blaauw et al. 2017; Nielsen et al. 2016), making it difficult to pinpoint the stage that attracted *H. halys*.

To determine whether *H. halys* indeed discriminates between plant phenological stages, we selected (dwarf) sunflower, which is a preferred host plant of *H. halys* (Nielsen et al. 2016; Soergel et al. 2015), and ran a laboratory four-choice experiment. Offering adult females a choice of plants in vegetative, pre-bloom, bloom, and seed stages, and recording the number of females present on these plants as the criterion for the choice of plant, we found that more females (45%) chose blooming sunflowers than preblooming and seeding sunflowers (22–28%) and that hardly any females (<5%) were present on vegetative-stage plants (Fig. 2). This degree of host plant discrimination by *H. halys* females was not mirrored in their choice of oviposition site. Egg masses were rather evenly distributed between all four plant stages (Fig. 2), suggesting that cues other than plant phenological stage affect oviposition choice by females, or that females "relied" on the high mobility of their offspring to switch between plants which were all located in close proximity in this experiment (Fig. 2).

Halyomorpha halys females sensed and behaviorally responded to sunflower odorants in olfactometer bioassays (Figs. 3 and 4) but the data did not completely align with the settling choices recorded in experiment 1 (Fig. 2). For example, vegetative-stage plants which were least often settled onto by females in experiment 1 strongly attracted females in experiment 2 (Fig. 2). These data indicate that different or additional cues may guide *H. halys* in the process of locating and accepting plants at a preferred physiological stage. At long range, it is generally the semiochemical profile of plants that guides foraging insects, particularly in visually complex habitats (Bruce et al. 2005; Webster and Cardé 2017). If also true for *H. halys*, this would explain why plant odor alone in the absence of visual plant cues attracted *H. halys* in olfactometer experiments (Figs. 3 and 4). At close range, visual plant cues such as size, shape, and color (Finch and Collier 2000; Rojas and Wyatt 1999), and even polarized light reflections from foliage (Blake et al. 2019), provide additional information and likely modulate the approach trajectory (Blake et al. 2020). After alighting on a potential host plant, acceptance and onset of feeding are then contingent upon plant tactile and gustatory cues which must have been poor on vegetative-stage sunflowers which prompted few settling responses by *H. halys* females (Fig. 2).

Based on plant odor alone, there was no one phenological stage of sunflower that was most attractive to *H. halys*, but blooming plants had the "edge" when taking all data into account. Blooming plants were: (*i*) significantly more attractive than seeding plants (Fig. 4, Exp. 11); (*ii*) somewhat more attractive than vegetative plants (Fig. 4, Exp. 7); (*iii*) as attractive as pre-bloom plants (Fig. 4, Exp. 9); and (*iv*) they were never repellent unlike pre-bloom and seeding plants (Fig. 3).

The odor profile among the phenological stages of sunflower differed markedly (Table 1), indicating that they could inform (long-range) foraging decisions by *H. halys*. As plants transitioned from pre-bloom to bloom, monoterpenes (α-pinene, β-pinene, camphene, sabinene), bornyl acetate, *E*3,*E*7-4,7-dimethyl-1,3,7-nonatriene and *E*3,*E*7-4,8,12-trimethyl-1,3,7,11-tridecatetraene increased in abundance 2- to 27-fold. Moreover, borneol being absent in the headspace of vegetative and pre-bloom plants appeared, albeit in small quantity. As the amount of nearly all of these odorants increased even further in seeding plants (Table 1), these odorants on their own are not likely signature semiochemicals of the blooming stage. The tridecatetraene, however, may serve such a signature role because it was prevalent only in the odor bouquet of blooming plants. The importance of this tridecatetraene for *H. halys* attraction could be tested by offering blends with or without it.

The synthetic blend of sunflower odorants for testing the attraction of *H. halys* in field settings was prepared according to the composition, amount, and ratio of odorants in headspace odorant extracts of blooming sunflower (Table 1). The blooming stage was selected for synthetic blend preparation because this plant stage, relative to other stages: (*i*) prompted the most settling responses by females in experiment 1 (Fig. 2); (*ii*) elicited overall more convincing attraction of females to odor sources in olfactometer experiments (Figs. 3 and 4); and (*iii*) produced the most distinct odor profile (Table 1). Moreover, if foraging *H. halys* were to exploit also plant visual cues, it would likely be the bright yellow petals of blooming sunflower that enhance the attractiveness of sunflower semiochemicals. With compelling evidence that the synthetic odorant blend attracted *H. halys* in olfactometers and that mineral oil could serve as a dissemination medium (Fig. 6), we proceeded to test the synthetic blend in field settings. Surprisingly, the synthetic blend on its own as a trap lure did not attract *H. halys*, but it significantly enhanced the attractiveness of synthetic *H. halys* pheromone, particularly in spring (Fig. 7).

Failure of the synthetic odor lure on its own to attract *H. halys* in field settings was likely due to the absence of plant visual cues and the presence of competing live host plants with complex semiochemical and visual cues. That both visual and olfactory plant cues guide foraging herbivores have been demonstrated in diverse insect taxa, including apple maggot flies (Epsky and Heath 1998; Morrison et al. 2016a), cabbage moths (Rojas and Wyatt 1999), and woodboring insects (Campbell and Borden 2009).

Our finding that a combined lure of *H. halys* pheromone and sunflower semiochemicals attracted more adult *H. halys* than pheromone alone (Fig. 7) implies that *H. halys* commonly communicate while residing on host plants. The many examples of plant odorants and aggregation or sex pheromones additively or synergistically attracting foraging insects include *Rhynchophorus* palm weevils (e.g., Gries et al. 1994), bark beetles (Deglow and Borden 1998), and fruit flies (Foster and Harris 1997).

The effectiveness of the combined *H. halys* pheromone and sunflower semiochemical lure for attraction of *H. halys* adults was most evident in the spring (Fig. 7) but became less apparent towards the fall when nymphs are more abundant and both adults and nymphs shift from plant foraging to overwintering, likely induced by a shortening photophase (Fig. 6, Exps. 16, 17). The attractiveness of this “combination lure” in spring could potentially be exploited for *H. halys* monitoring and management. For example, effectively attracting *H. halys* in spring to (plant) surfaces treated with contact insecticide would help prevent population build-up and thus curtail crop damage throughout the growing season. Such a tactic, however, would become economically more viable if the currently complex sunflower semiochemical blend (Table 1) could be reduced to a few essential constituents without diminishing its attractiveness to *H. halys*. Future research will determine whether the goal of developing a simple and effective sunflower semiochemical lure is attainable.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10886-021-01281-y.

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Author Contributions GG, PA, and WW conceived the study; WW captured headspace plant odorants and ran laboratory olfactometer and field experiments; RG analyzed plant odorants; SA synthesized chemicals; WW and PA analyzed data; WW and GG wrote the first draft and all authors reviewed and approved of the final draft.

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Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication All authors approved of the submission of the manuscript.

Conflict of Interest The authors declare no conflicts of interest.

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