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Behavioural responses of wheat stem sawflies to wheat volatiles

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Abstract

1 Adult wheat stem sawflies *Cephus cinctus,* pests of cultivated cereals that also infests wild grasses, migrate into wheat fields where they oviposit in elongating, succulent stems.

2 Volatiles released by wheat plants at susceptible stages were analyzed to determine potential semiochemical compounds. Seven major compounds were identified and quantified.

3 A Y-tube bioassay was developed to evaluate upwind orientation of adult sawflies in response to an airstream that passed over elongated wheat plants. The bioassay was also conducted with synthetic volatile compounds. The compounds were tested using a range of concentrations spanning those identified in the airstream passing over wheat plants.

4 A significant number of adult females were attracted to wheat plants when given a choice of either purified air or the air passing over plants.

5 A significant number of female *C. cinctus* were attracted to (*Z*)-3-hexenyl acetate, β-ocimene, and (*Z*)-3-hexen-1-ol, but were repelled by 6-methyl-5-hepten-2-one. Females did not respond to (*E*)-2-hexenal, or (*E*)-2-hexenyl acetate. The behavioural responses were concentration dependent; the highest tested concentration of (*Z*)-3-hexenyl acetate was repellent to females of this species.

6 Adult males did not discriminate between air passing over wheat plants and air from a purified airstream. Males did not respond to any tested synthetic compound at any concentration.

7 The present study demonstrates for the first time that adult females of wheat stem sawfly display innate behaviours in response to synthetic volatiles. These results provide a basis for the potential development of resistant wheat varieties and for the development of semiochemically-based pest management.

Keywords *Cephus cinctus*, insect behaviour, semiochemicals, *Triticum aestivum*, volatiles, wheat, wheat stem sawfly, Y-tube olfactometer.

Introduction

The wheat stem sawfly *Cephus cinctus* Norton (Hymenoptera: Cephidae) has been a recognized pest of wheat *Triticum aestivum* L. (Cyperales: Poaceae) in the northern Great Plains of North America for almost a century. Infested plants produce lower yields and usually lodge, reducing the amount of grain that can be harvested (Ainslie, 1920; Holmes, 1977; Morrill *et al.*, 1992). This insect cannot be managed using insecticides and solid-stem cultivars are only partially resistant (Weaver *et al.*, 2004). The larvae overwinter in below-ground portions of stems, emerging as adults late in the spring during crop growth. Males appear first, with most mating occurring during the first day of adult female emergence. Females mate once and then spend the rest of their brief lifespan searching for and ovipositing in large-stemmed graminous hosts (Ainslie, 1920; Weiss *et al.*, 1992). The pest is univoltine with a flight period that varies from slightly longer than

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1 week to approximately 1 month (Morrill & Kushnak, 1999). Rapid location and selection of suitable hosts is paramount because adult sawflies live less than 7 days post-emergence (Morrill *et al.*, 2000). An additional limitation on successful oviposition is the need to deposit an egg in rapidly-growing, young, succulent green shoots. There is a narrow time window when plants are susceptible to attack, and most eggs are deposited between Zadoks growth stages 32 and 49 (Zadoks *et al.*, 1974), which comprise the initiation of stem elongation and the emergence of the inflorescence, respectively (Holmes & Peterson, 1960).

Volatile compounds produced by wheat have been collected and identified using techniques ranging from steam-distillation and reduced pressure extraction to collection of entrained volatiles from seedlings starting at the two-leaf stage (Hamilton-Kemp & Anderson, 1984, 1986; Buttery *et al.*, 1985; Batten *et al.*, 1995; Quiroz *et al.*, 1997; Quiroz & Niemeyer, 1998) through to the wheat panicles (Birkett *et al.*, 2004). The characteristic 'green' odour of wheat is due to eight, volatile, 6-carbon aldehydes and alcohols that are metabolized via the lipoxygenase pathway from α-linolenic and linoleic acids (Hatanaka, 1993), although terpenes and other volatile compounds have been reported to be released by wheat as well (Quiroz & Niemeyer, 1998; Runyon *et al.*, 2006).

At present, more than 1000 low-molecular-weight organic compounds have been reported to be emitted from plants, including alkanes, alkenes, alcohols, ketones, aldehydes, ethers, esters and carboxylic acids (Dudareva *et al.*, 2004, Niinemets *et al.*, 2004). One alternative to the use of synthetic insecticides is deployment of semiochemicals utilized by insect herbivores in host selection (Martel *et al.*, 2005b). Some studies have demonstrated that adults of *Phyllopertha horticola* can be captured in traps containing (*Z*)-3-hexen-1-ol (Ruther & Tolasch, 2004). Moreover, successful mass trapping using synthetic pheromones and plant co-attractants has been reported for *Rhynchophorus* spp., *Oryctes* spp. and *Scapanes australis* (Oehlschlager *et al.*, 2002). The use of host plant compounds that act as semiochemicals influencing insect behaviour is a promising method of crop protection (Agelopoulos *et al.*, 1999; Reddy *et al.*, 2004; Martel *et al.*, 2005a). Exploitation of semiochemicals for pest management can be achieved using various strategies. Through crop diversification and intercropping, naturally-occurring plant semiochemicals can be used to manipulate insect behaviour (Banks & Ekbom, 1999; Khan *et al.*, 2006). Trap cropping can take advantage of insect–plant interactions by augmented amounts of semiochemicals using a 'push–pull' or stimulo-deterrent strategy where repellent and attractive stimuli are deployed in tandem to manipulate the distribution of insect pests, reducing reliance on insecticides (Miller & Cowles, 1990; Cook *et al.*, 2007). Plant compounds can be used in traps alone (Katsoyannos & Guerin, 1984; Martel *et al.*, 2005a, b; Ruther & Mayer, 2005) or to synergize the effect of pheromones in trap catches (Nakamuta *et al.*, 1997; Reddy *et al.*, 2004; Leskey *et al.*, 2005) for mass trapping, monitoring or in attracticide strategies. Another potential use of semiochemicals in pest management is application of volatile compounds that act as repellents (Pettersson *et al.*, 1994; Borden *et al.*, 1997). Some plant semiochemicals have been found to induce defence responses in plants, which are recognized by insects. Application of one of these chemicals, methyl salicylate, has been shown to provide protection from aphids in treated wheat (Pettersson *et al.*, 1994). It has also been proposed that semiochemicals could potentially be used to enhance biocontrol by using prey compounds to attract parasitoids to target areas (Nakashima *et al.*, 2004). Plants could be genetically engineered to activate defense genes that would aid in plant-plant signalling (Pickett *et al.*, 2003) or to switch on the release of herbivore-damaged volatiles that may attract parasitoids (Kappers *et al.*, 2005).

An understanding of insect–plant interactions and chemical ecology is needed for the success of management practices relying on semiochemicals (Pickett *et al.*, 1997; Cook *et al.*, 2007). The study of the interaction between host volatiles and behaviour in the wheat stem sawfly could lead to the development of synthetically-baited lures to be used in field traps, possibly in combination with identified pheromone compounds (Cossé *et al.*, 2002). It may also provide new approaches to applied host-plant resistance (Buttery *et al.*, 1985) that could include nonpreference (Pedigo, 2002) in conjunction with trap cropping, particularly by exploiting innate varietal variability.

In the present study, we identified volatiles produced by a cultivar of wheat commonly grown in Montana. The volatiles were collected from plants during stem elongation, the predominant stage at which plants are susceptible to sawfly infestation in the field due to the synchrony between adult flight and crop phenology. We conducted behavioural experiments to test the response of sawflies to some of these volatiles as a means to gain further insight into the host seeking behaviour of the sawfly and to identify potential attractants or repellents that may aid in management of this insect pest.

Materials and methods

Source and handling of insects

Overwintering wheat stem sawfly larvae were collected in postharvest stubble. Random stubble samples were collected throughout a wheat field in Broadwater County, Montana. Infested, 'sawfly-cut' stems were collected and sorted into lots of 100. These were stored in plastic Ziploc bags and held at 4 °C for $>$ 100 days in the dark to facilitate completion of obligatory larval diapause (Holmes, 1977). Single stems were then placed in three-dram shell vials sealed with cotton plugs. Vial plugs were lightly wetted twice weekly with distilled water to prevent desiccation of the developing larvae. Vials were held at room temperature (22–27 °C) under a photoperiod in the range LD 12 : 12 h to 15 : 9 h. The adults generally emerged in 4–5 weeks. Vials were examined each morning, and those containing a newly-emerged adult were held in a darkened box until trials were initiated. All bioassays were conducted with unmated adults within 24 h of eclosion from the sawfly-cut stem.

Plant material

Experiments were performed at the Plant Growth Center at Montana State University, Bozeman. Hard red spring wheat (cv. McNeal) seeds were sown in a greenhouse with supplemental light (GE Multi-Vapor Lamps -model MVR1000/C/U, GE Lighting, General Electric Co., Cleveland, Ohio), controlled temperature and ambient humidity, under LD 15 : 9 h photoperiod. Daytime temperature was 22 ± 2 °C and the overnight temperature was 20 ± 2 °C. The relative humidity typically was in the range 20–40% with infrequent increases due to rare, sustained precipitation events outside the facility. Plants were grown in equal parts of MSU PGC soil mix (equal parts of sterilized Bozeman Silt Loam soil: washed concrete sand and Canadian sphagnum peat moss) and Sunshine Mix #1 (Canadian sphagnum peat moss, perlite, vermiculite, and Dolomitic lime; Sun Gro Horticulture, Inc., Bellevue, Washington). The plants were watered four times weekly, and fertilized with Peters General Purpose Fertilizer (J. R. Peters Inc, Allentown, Pennsylvania) at 100 p.p.m. in aqueous solution twice each week, as part of the regular watering schedule. Fertilizer application commenced when the plants reached the third leaf stage.

Volatile collection

Volatiles were collected from plants before the emergence of the inflorescence from the sheath at the tip of the stem (Zadoks 41–49) and the main volatile compounds produced were identified. Stems with emerging inflorescences were chosen for experimentation because it is the predominant stage targeted for oviposition by female sawflies (Ainslie, 1920; Holmes & Peterson, 1960). The mean ± SE weight of the plants was 8.54 ± 0.44 g.

To conduct behavioural bioassays using whole wheat plants, it was necessary to remove the plants from the pots. Given that potential wounding during uprooting may cause changes in the volatile production of plants, we compared volatile compounds produced by intact and uprooted plants to assess potential changes in volatile production induced by uprooting. To minimize stress responses, we gently uprooted the plants and used them in the bioassays immediately afterwards, as has been reported for excised or manipulated tissue by other authors (Gols *et al.*, 2003). Wheat plants were carefully pulled from the soil and the roots were wrapped with wet cotton for imminent volatile collection (Turlings *et al.*, 1991, 1993, 2000). Other plants used in these experiments were left intact and growing in the pots. Volatiles from the intact as well as from the uprooted plants were collected for 8 h between 10.00 and 18.00 h (combined ambient and supplemented photophase). Two replicates were conducted, each with six intact and six uprooted plants.

To analyze volatiles emitted by wheat that might have behavioural activity, plants were placed in collection chambers in a volatile collection system as previously described by Piesik *et al.* (2006). The apparatus featured 12 glass volatile collection chambers (diameter 40 mm, length 800 mm) attached to a volatile collection port on one end and open on the other end to enclose one individual plant per chamber. A Teflon sleeve enclosed the base of the stem and was taped to the glass tube to prevent outside air from entering the system. A volatile collector trap (long glass tube: outer diameter 6.35 mm, length 76 mm; Analytical Research Systems, Inc., Micanopy, Florida) containing 30 mg of Super-Q (Alltech Associates, Inc, Deerfield, Illinois) adsorbent was inserted into each volatile collector port. Purified, humidified air was delivered at a rate of 1.0 L/min over the plants, and the flow and pressure were maintained by a regulated vacuum pump.

Analytical methods

Volatile chemicals were eluted from the traps with 200 μL of certified ACS Spectranalyzed hexane (Fisher Scientific, Fair Lawn, New Jersey). Ten μL of a 0.73 ng/μL of a solution of trans-2-nonene in hexane was added as internal standard, and then 3 μL of the sample were transferred to the gas chromatograph (GC) column (HP-5MS; 30 m × 0.25 mm, 0.25 μm film thickness; J&W Scientific, Flosom, California). The system used to analyze the volatiles was a GC (Agilent 6890 instrument; Agilent Technolgies, Santa Clara, California) coupled to a mass selective detector (MSD, Agilent 5973 instrument). The samples were injected onto the column in pulsed-splitless mode, with an initial pressure of 82.7 kPa for 1 min. The inlet temperature of the GC was set at 250 °C. The column temperature was held at 50 °C for 4 min and then increased in temperature at a rate of 5°C/min until it reached 160 °C; at this temperature, the rate of increase changed to 25 °C/min until a final temperature of 280 °C. A temperature of 300 °C was maintained for the transfer line to the MSD. The flow rate in the column was set at 1.2 mL/min.

The identities of volatile compounds of interest were determined from the comparison to the mass spectra and retention times of authentic standards. Quantification of compounds was made relative to the internal standard. Both isomers of β-ocimene were present in commercial (*Z*)-β-ocimene [70% (*Z*)-β-ocimene in 25% (*R*)-(+)-limonene; Fluka, Switzerland] and were used to quantify production of these isomers by the experimental wheat plants.

The quantified volatile data were square-root transformed to better meet the homogeneity of variance assumption. Differences in volatile production between intact and uprooted plants were analyzed using analysis of variance (PROC GLM; SAS, 1998).

Y-tube olfactometer

There were seven compounds that were consistently detected in the volatile collections. Bioassays were conducted to test behavioural activity of volatiles released by wheat plants and of the synthetic compounds. The Y-tube system used was similar to that described and illustrated previously by Daisy *et al.* (2002). A charcoal-purified and humidified air stream was connected to each arm of the Y-tube by a threaded 24/410 (inner diameter 24 mm) cap with a Teflon liner coupled to a 0.64-cm Swagelock union delivering air via Teflon tubing (outer diameter 0.64 cm). The airflow was set at 0.8 L/min using a flowmeter. The synthetic or plant test-stimulus was placed in either a synthetic lure (outer diameter 24 mm,

length 8 cm) or a Corning tube (outer diameter 24 mm, length 46 cm; for one entire wheat plant), terminating in a male ground-glass joint at one end, with a threaded-glass joint to receive the 24/410 threaded air supply cap at the other end. A wire screen to hold the test stimulus was embedded at 4.5 cm from the distal end of this tube, 0.5 cm above the beginning of the male ground-glass joint.

The Y-tube olfactometer comprised Corning glass tubing (outer diameter 28 mm, length 30 cm) that branched at 20 cm. The interior angle of the 'Y' was 120°, and the diverging arms extended for 4 cm in each direction before becoming parallel for their final 10 cm, and then terminated in a female ground-glass joint at the end of each arm. The male ground glass joint on the stimulus-delivery tube was inserted into the receiving arm of the Y-tube, yielding a consistent airtight fit.

Bioassay subjects were placed in the unbranched section of the Y-tube at approximately 2 cm from the outlet. It was necessary to place a 28-cm long wire in the bottom of the tube, extending from the introduction point to the junction of the 'Y' to facilitate subject movement toward the test junction. Positive phototaxis was additionally employed to enhance insect movement. A 6.3 V bulb (lamp type #46; Radio Shack, Fort Worth, Texas) was placed between the apexes of the Y-tube arms; equality in illumination supplied to the two arms was verified using a pyranometer (model LI-200SA; LI-COR, Inc., Lincoln, Nebraska).

For experiments using whole plants in the Y-tube olfactometer, wheat was grown until it was in the Zadoks 41–49 growth stages. The plants were gently uprooted, and the roots were washed in room temperature water and wrapped in moist cotton as described above for the collection of volatiles. An equivalent amount of moist cotton was placed in the control arm of the Y-tube. Each plant was used only once, for a bioassay lasting up to 7 min after the plant was uprooted.

For each bioassay, a single insect was placed on the wire in the base of the Y-tube. Insects were observed for 7 min, or until they moved upwind along the wire and into one of the arms of the Y-tube that carried either the airborne volatile chemicals or the control stream of purified air. One hundred and forty insects of each sex were used with plant material as the test stimulus. Fifty insects of each sex were used for each concentration of all tested compounds. Statistical analyses were performed using a chi-square test for small sample sizes (Sokal & Rohlf, 1995).

Synthetic chemicals

Commercial samples of the volatile compounds were obtained from Sigma-Aldrich Chemical Co. Inc. (Milwaukee, Wisconsin) [(*Z*)-3-hexenol, (*E*)-2-hexenal, 6-methyl-5-hepten-2-one, (*E*)-2-hexenyl acetate, (R)-(+)-limonene - stabilizer for β-ocimene]; Bedoukian Research Inc. (Danbury, Connecticut) [(*Z*)-3-hexenyl acetate] and Fluka (Switzerland) [70% (*Z*)-β-ocimene in 25% (R)-(+)-limonene; in which the actual amount of (*Z*)-β-ocimene was 49.7%, and the preparation also contained 0.4% (*E*)-β-ocimene].

The limonene present in the synthetic preparations as a stabilizer was also tested in isolation for activity. The tested concentration of stabilizer was equal to that present in the behaviourally active preparation of the synthetic wheat compound provided by the commercial supplier. This test would determine whether any behavioural activity due to the stabilizer might impact the biologically relevant concentration of the wheat volatile.

Release and calibration of volatiles

To screen behavioural activity of the volatile compounds identified from wheat plants, aliquots of commercially-available compounds were used at seven incrementally increasing concentrations. The range of concentrations used in the bioassays spanned the mean amounts produced by individual plants found in the present study. The release rates for the various preparations tested are given in Table 1.

The compounds were diluted in hexane and added to one quarter of a 55-mm disk of Whatman No. 1 filter paper (Whatman International Ltd, U.K.) folded inside a microcentrifuge tube (Fisher Scientific, Pittsburgh, Pennsylvania). Manipulation and control of the desired rates of release of synthetic wheat volatiles for bioassays were achieved by varying the concentration of the hexane solution applied to the filter paper substrate within a microcentrifuge tube, and by varying the number of holes in the cap of the 2.0-mL flat-top microcentrifuge tube enclosing the filter paper. One or more holes were made in the cap to facilitate release of the volatile stimulus by using either a standard push pin (Stockwell Office Products, Framingham, Massachusetts) or a 27.5-gauge syringe needle (Allison Medical Inc, Englewood, Colorado). The microcentrifuge cap was left open for 1 min to allow the evaporation of the hexane and up to 5 min to achieve the desired release rates.

Release rates, over a 7-min collection that matched the bioassay duration, were quantified and calibrated using the volatile collection system and coupled gas chromatograph-mass spectrometer as previously described. A filter paper was treated with hexane in a microcentrifuge tube, evaporated for 1 min with the cap open, and prepared for use as a control stimulus for each trial. Before each bioassay, all glassware, pins, syringe needles and bioassay wires were cleaned thoroughly with detergent, followed by repeated rinses with acetone, and then repeated rinses with hexane. After solvent rinses, the glassware was stored at 80 °C for at least 1 h before use.

Results

Volatiles emitted by wheat plants

The seven volatile compounds collected from wheat plants at emergence of the inflorescence included the green-leaf volatiles (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate, (*E*)-2-hexenal and (*E*)-2-hexenyl acetate, plus 6-methyl-5-hepten-2-one, and the terpenes (*E*)-β-ocimene and (*Z*)-β-ocimene. Univariate analyses of variance revealed that intact plants released lower amounts of (*Z*)-3-hexenyl acetate than uprooted plants, ($F = 5.41$, d.f. $= 1, 11$, $P = 0.04$) and showed a trend toward slightly lower amounts of (*Z*)-β-ocimene

Table 1 Release rates, over a collection period of 7 min, for volatile compound treatments used in behavioural assays

Behaviourally active compounds			Behaviourally inactive compounds		
Compound	Lure dose (ng)[a]	Release rate, ± SEM (ng/h)	Compound	Lure dose (ng)[a]	Release rate, ± SEM (ng/h)
(*Z*)-3-hexen-1-ol	9	0.24 ± 0.06	(*E*)-2-hexenal	8	0.12 ± 0.012
	17[b]	0.6 ± 0.06		32	0.36 ± 0.024
	17[c]	1.2 ± 0.36		42	0.48 ± 0.048
	42[d]	2.4 ± 0.36		85	0.96 ± 0.12
	212[e]	6 ± 1.2		212	2.4 ± 0.36
	423[f]	12 ± 4.8		423	4.8 ± 1.2
	4230[f]	120 ± 36		4230	48 ± 3.6
6-methyl-5-hepten-2-one	9	0.06 ± 0.012	(*E*)-2-hexenyl acetate	27	0.12 ± 0.012
	32	0.3 ± 0.054		103	0.36 ± 0.12
	43	0.42 ± 0.06		135	0.48 ± 0.12
	86	0.6 ± 0.12		270	1.2 ± 0.12
	214	1.8 ± 0.6		674	2.4 ± 0.24
	428	4.2 ± 1.2		1350	4.8 ± 0.36
	4280	42 ± 5.4		13500	156 ± 1.2
(*Z*)-3-hexenyl acetate	450	18 ± 4.2			
	1795[c]	60 ± 4.2			
	4485[f]	120 ± 18			
	8970	180 ± 24			
	13455[g]	420 ± 24			
	22425[h]	840 ± 360			
	179400[h]	8400 ± 150			
β-ocimene	27	0.12 ± 0.012			
	40	0.36 ± 0.024			
	82	0.48 ± 0.048			
	205	0.96 ± 0.12			
	410	2.4 ± 0.36			
	4100	4.8 ± 1.2			
	8000	48 ±3.6			

[a]All holes were made with a standard push pin. Solvent was evaporated for 1 min before testing, unless otherwise noted.
[b]One hole was made with 27.5-gauge syringe needle, with a 3-min evaporation interval before testing.
[c]Two-minute evaporation interval before testing.
[d]Three holes.
[e]Three-minute evaporation interval before testing.
[f]Five-minute evaporation interval before testing.
[g]Two holes, 2-min evaporation interval before testing.
[h]Four holes, 2-min evaporation interval before testing.

($F = 3.28$, d.f. = 1, 11, $P = 0.097$) (Table 2). The slight increase in volatile compounds released by uprooted plants was probably the result of a wound response to uprooting, which releases C_6-volatiles (Bate & Rothstein, 1998; Turlings & Benrey, 1998). (*E*)-β-ocimene has also been reported to be induced by mechanical wounding (Fäldt *et al.*, 2003).

Behavioural assays

Air passing over freshly-uprooted wheat plants elicited rapid upwind orientation in responding female sawflies within 5 min of the 7-min bioassays ($\chi^2 = 8.89$, $P < 0.005$, $n = 138$ of 140 responding; 87 responding to air entrained with wheat volatiles). Male sawflies did not show any preference for either air passing over wheat plants or the control airstream in Y-tube bioassays of equivalent duration ($\chi^2 = 0.38$, $P > 0.5$, $n = 130$ of 140 responding, 63 responding to air entrained with wheat volatiles).

Manipulation and control of the solvent and dose of the synthetic compounds applied to filter papers allowed for consistent, known release rates from the treated filter papers in the Y-tube bioassays, which is a critical and often overlooked step in using synthetic volatile compounds in semiochemical assays (Table 1). Female sawflies were attracted to (*Z*)-3-hexenyl acetate at airborne concentrations of 60, 120 and 180 ng/h and were repelled at 8.4 μg/h (Tables 1 and 3). Females also were attracted to airborne β-ocimene at 0.96 ng/h, and to (*Z*)-3-hexen-1-ol at 6 ng/h (Tables 1 and 3). Repellency was observed for females exposed to 6-methyl-5-hepten-2-one at 42 ng/h (Tables 1 and 3). Females did not respond significantly to other tested concentrations of these compounds with behavioural activity (Table 3), nor did they respond significantly to any tested concentration of (*E*)-2-hexenal or (*E*)-2-hexenyl acetate. Male responses were not statistically different from the control for any compound, at any concentration (Tables 1 and 3). The stabilizer (R)-(+)-limonene that was a component in certain synthetic

Table 2 The amount of volatile compounds in the odor blend quantified for intact and uprooted wheat plants

Compound	Intact plants (ng/h)	Uprooted plants (ng/h)
(*E*)-2-hexenal	0.003 ± 0.001	0.01 ± 0.007
(*Z*)-3-hexen-1-ol	0.16 ± 0.1	0.11 ± 0.05
6-methyl-5-hepten-2-one	0.38 ± 0.02	0.41 ± 0.04
(*Z*)-3-hexenyl acetate	1.52 ± 0.22	4.50 ± 1.66
(*E*)-2-hexenyl acetate	0.17 ± 0.05	0.20 ± 0.08
(*E*)-β-ocimene	32.17 ± 8.58	38.28 ± 9.27
(*Z*)-β-ocimene	1.24 ± 0.11	1.56 ± 0.17

Data are the amount per plant (mean ± SEM) ($n = 12$ for each treatment, uprooted or intact plants).

preparations showed no behavioural activity in isolation at the tested concentrations.

Discussion

This is the first demonstration that female wheat stem sawflies utilize plant volatiles to locate suitable hosts. Rapid host location is critical for this species, which has a comparatively brief flight period that is synchronized with stem elongation. Female sawflies responded positively to volatile chemicals collected from wheat plants of a cultivar that is highly attractive and commonly infested in the fields. However, (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol and 6-methyl-5-hepten-2-one caused behavioural activity at concentrations that were higher than those released by wheat plants. A possible answer for this could be that for attraction or repellency to occur at volatile concentrations characteristic from plants, additional factors are required, such as exposure to blends of compounds in the same ratios as those produced by plants (Birkett *et al.*, 2004; Bruce *et al.*, 2005). In the present study, the volatile compounds were tested individually. In addition, the release rates from the filter papers in the Y-tube apparatus and those from intact plants may not be directly comparable.

The commercially-available mixture of β-ocimene isomers was attractive to female sawflies. In the present study, wheat plants were found to produce mainly (*E*)-β-ocimene, whereas the (*Z*)-β isomer was produced in smaller amounts. Given that it is not possible to obtain pure (*Z*)- or (*E*)-β-ocimene, we cannot be certain of the relative behavioural activity of the two isomers of this molecule produced by wheat plants. Gas chromatography coupled–electroantennography (GC–EAD) experiments will be conducted to determine this. However, it is possible that the wheat stem sawfly antennae can detect both isomers of the molecule, as was observed in heliothine moths of which the most sensitive olfactory receptor neurones respond secondarily to another pheromone that is chemically related (Almaas *et al.*, 1991).

We observed neither positive or negative responses of male sawflies to any of the tested wheat volatiles, and the differences between the responses of the male and female insects may be explained by the need to fly upwind from fallow emergence sites, a characteristic of this cropping system, to find host plants for oviposition (Nansen *et al.*, 2005). The wheat stem sawfly is a major pest in a dryland production system that features alternate-year, summer-fallow cropping. This practice results in sawfly overwintering sites remaining unplanted for the next year, so short-lived adults must actively fly to nearby crop stands (Weaver *et al.*, 2004). Males remain at locations where encounters with females are likely (Cossé *et al.*, 2002) but females have the task of searching among host plants for stems in which they can oviposit (Weaver *et al.*, 2005). Adult wheat stem sawflies do not feed, so locating suitable hosts might not be a priority for males. Moreover, the mating system of wheat stem sawflies involves the formation of nonresource based leks, in which males usually aggregate on the edges of wheat fields (D. K. Weaver, unpublished data). Therefore the antennal receptors of males may be specialized for pheromones rather than for plant compounds.

The results obtained in the present study are encouraging given the ongoing search for an attractant for wheat stem sawfly females. Efforts are underway to identify biologically relevant isomers and other potential semiochemicals released by host plants using GC–EAD. When all active compounds in the volatile collections from host plants are identified, detailed behavioural studies will be undertaken with blends containing varying amounts of behaviourally active components. These experiments will determine the optimal blend of the synthetic plant compounds for use in applied research targeting semiochemical based management.

Pheromones involved in wheat stem sawfly mating have been identified (Bartelt *et al.*, 2002; Cossé *et al.*, 2002) and have been used as lures in provisional field traps designed to monitor flight activity (Cossé *et al.*, 2002). Investigation of the potential synergistic activity between plant and insect volatiles in field lures is underway. An attracticide that includes pheromones as well as chemicals that attract females is likely to be more effective than one using pheromones alone (Foster & Harris, 1997).

The results from the present study will provide a potential basis for development of resistant wheat varieties based on the concept of resistance by nonpreference by identifying or breeding cultivars that are unattractive or cannot be recognized by female sawflies (Smith, 1989; Pedigo, 2002). This is particularly compelling because the wheat stem sawfly cannot be effectively suppressed using conventional agricultural methods, including insecticides (Weiss *et al.*, 1992; Weaver *et al.*, 2004). As a univoltine species that has a short adult flight period and stage-specific oviposition requirements, it is particularly suited for targeted semiochemical intervention. The potential of implementing semiochemical based management that could be readily integrated with innate biological control from endemic, native braconid parasitoids (Runyon *et al.*, 2002; Weaver *et al.*, 2005) presents a unique opportunity to manage this chronic, severe pest.

Manipulation of semiochemicals could also aid in the use of trap cropping to manage this insect. Through conventional plant breeding or by genetic engineering (Pickett *et al.*, 1997), wheat may be developed to release increased amounts of volatiles that attract ovipositing females. Furthermore, application of slow release formulations of a blend of attractive chemicals may be used to concentrate ovipositing females in a reduced area of the field (Martel *et al.*, 2005a, b). However,

Table 3 Responses of adult wheat stem sawflies to wheat plant volatiles in a Y-tube olfactometer

Compound	Lure release rate ± SEM (ng/h)	Males				Females			
		+	−	NR	χ^2	+	−	NR	χ^2
(*Z*)-3-hexenyl acetate	18 ± 4.2	20	26	4	0.54	23	27	0	0.18
	60 ± 4.2	26	24	0	0.02	36	14	0	8.82[a]
	120 ± 18	24	25	1	0	41	9	0	19.22[a]
	180 ± 24	22	26	2	0.18	33	17	0	4.5[a]
	420 ± 24	18	29	3	2.12	31	19	0	2.42
	840 ± 360	23	24	3	0	22	28	0	0.5
	8400 ± 150	21	27	2	0.52	10	40	0	16.82[a]
β-ocimene	0.12 ± 0.012	20	26	4	0.54	24	26	0	0.02
	0.36 ± 0.024	22	25	3	0.08	30	18	2	2.52
	0.48 ± 0.048	24	22	4	0.02	28	22	0	0.5
	0.96 ± 0.12	20	24	6	0.2	34	16	0	5.78[a]
	2.4 ± 0.36	25	22	3	0.08	32	18	0	3.38
	4.8 ± 1.2	20	29	1	1.3	20	30	0	1.62
	48 ±3.6	23	24	3	0	26	24	0	0.02
(*Z*)-3-hexen-1-ol	0.24 ± 0.06	21	27	2	0.52	22	28	0	0.5
	0.6 ± 0.06	23	24	3	0	27	23	0	0.18
	1.2 ± 0.36	26	22	2	0.18	25	25	0	0.02
	2.4 ± 0.36	22	23	5	0	27	23	0	0.18
	6 ± 1.2	20	26	4	0.54	37	13	0	10.58[a]
	12 ± 4.8	22	25	3	0.08	32	18	0	3.38
	120 ± 36	28	22	0	0.5	26	24	0	0.02
6-methyl-5-hepten-2-one	0.06 ± 0.012	21	28	1	0.7	28	22	0	0.5
	0.3 ± 0.054	22	26	2	0.18	29	21	0	0.98
	0.42 ± 0.06	23	25	2	0.02	20	29	1	1.3
	0.6 ± 0.12	23	25	2	0.02	27	21	2	0.52
	1.8 ± 0.6	24	23	3	0	26	24	0	0.02
	4.2 ± 1.2	20	23	7	0.09	20	27	3	0.76
	42 ± 5.4	19	25	6	0.56	15	33	2	6.02[a]
(*E*)-2-hexenal	0.12 ± 0.012	25	23	2	0.02	26	24	0	0.02
	0.36 ± 0.024	23	23	4	0.02	23	27	0	0.18
	0.48 ± 0.048	25	25	0	0.02	27	23	0	0.18
	0.96 ± 0.12	26	18	6	1.11	20	30	0	1.62
	2.4 ± 0.36	22	22	6	0.02	26	22	2	0.18
	4.8 ± 1.2	19	27	4	1.06	21	29	0	0.98
	48 ± 3.6	19	25	6	0.56	24	25	1	0
(*E*)-2-hexenyl acetate	0.12 ± 0.012	22	27	1	0.32	23	27	0	0.18
	0.36 ± 0.12	28	21	1	0.73	28	20	2	1.02
	0.48 ± 0.12	20	26	4	0.54	23	25	2	0.02
	1.2 ± 0.12	21	27	2	0.52	26	23	1	0.08
	2.4 ± 0.24	22	26	2	0.18	24	24	2	0.02
	4.8 ± 0.36	29	20	1	1.3	30	18	2	2.52
	156 ± 1.2	20	28	2	1.02	27	23	0	0.18

[a]Significant behavioural activity at $\alpha = 0.05$.
+, Number of individuals responding to lure; −, number of individuals responding to control arm; NR, number of individuals not responding ($n = 50$).

field application of volatiles is costly (Shelton & Badenes-Perez, 2006) and the emission of increased amounts of certain compounds might have added energetic and ecological costs to the plants (Turlings & Wäckers, 2004). Therefore, the use of a stimulo-deterrent strategy that takes advantage of naturally-occurring semiochemicals appears to be more feasible in the short term. As has been shown in maize, *Zea mays* L. (Gouinguené *et al.*, 2001), different genotypes are likely to vary in the emission of volatiles. A survey of volatiles released across selected wheat cultivars might reveal differences that could translate into differential attractiveness. Currently, research is being conducted to determine the release rate of these behaviourally active compounds from different wheat cultivars, as well as from other grass species that commonly occur in sawfly infested areas. The use of attractive cultivars as trap crops to protect unattractive cultivars or species can be further explored. Such a stimulo-deterrent control strategy has been successful in managing maize stem borers *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and *Busseola fusca* Fuller (Lepidoptera: Noctuidae) by intercropping unattractive crops with maize and surrounding the fields with an attractive trap crop (Khan & Pickett, 2004).

As discussed above, the results of the present study will contribute to the development of management tactics that rely on the exploitation of semiochemicals to manipulate oviposition behaviour of female sawflies, such as mass trapping,

trap cropping and, potentially, the development of new cultivars with varying degrees of attractiveness.

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References

Agelopoulos, N., Birkett, M.A., Hick, A.J, Hooper, A.M., Pickett, J.A., Pow, E.M., Smart, L.E., Smiley, D.W.M., Wadhams, L.J. & Woodcock, C.M. (1999) Exploiting semiochemicals in insect control. *Pesticide Science*, **55**, 225–235.

Ainslie, C.N. (1929) *The Western Grass-Stem Sawfly: A Pest of Small Grains. U.S. Department of Agriculture Technical Bulletin* No. 157; 24 pages.

Almaas, T.J., Christensen, T.A. & Mustaparta, H. (1991) Chemical communication in heliothine moths. I. Antennal receptor neurons encode several features of intra- and interspecific odorants in the male corn earworm moth *Helicoverpa zea*. *Journal of Comparative Physiology*, **169**, 249–258.

Banks, J.E. & Ekbom, B. (1999) Modelling herbivore movement and colonization: pest management potential of intercropping and trap cropping. *Agricultural and Forest Entomology*, **1**, 165–70.

Bartelt, R.J., Cossé. A.A., Petroski, R.J. & Weaver, D.K. (2002) Cuticular hydrocarbons and novel alkenediol diacetates from the wheat stem sawfly (*Cephus cinctus*): natural oxidation to pheromone components. *Journal of Chemical Ecology*, **28**, 385–405.

Bate, N.J. & Rothstein S.J. (1998) C_6-volatiles derived from the lipoxygenase pathway induce a subset of defense related genes. *Plant Journal*, **16**, 561–569.

Batten, J.H., Stutte, G.W. & Wheeler, R.M. (1995) Effect of crop development on biogenic emissions from plant populations grown in closed plant growth chambers. *Phytochemistry*, **39**, 1351–1357.

Birkett, M.A., Bruce, T.J.A., Martin, J.L., Smart, L.E., Oakley, J. & Wadhams, L.J. (2004) Responses of female orange wheat blossom midge, *Sitodiplosis mosellana*, to wheat panicle volatiles. *Journal of Chemical Ecology*, **30**, 1319–1328.

Borden, J.H., Chong, L.J. Savoie, A. & Wilson, I.M. (1997) Responses to green leaf volatiles in two biogeographic zones by striped ambrosia beetle, *Trypodendron lineatum*. *Journal of Chemical Ecology*, **23**, 2471–2479.

Bruce, T.J.A., Wadhams, L.J. & Woodcock, C.M. (2005) Insect host location: a volatile situation. *Trends in Plant Science*, **10**, 269–274.

Buttery, R.G., Xu, C. & Ling, L.C. (1985) Volatile components of wheat leaves (and stems): possible insect attractants. *Journal of Agriculture and Food Chemistry*, **33**, 115–117.

Cook, S.M., Khan, Z.R. & Pickett, J.A. (2007) The use of push-pull strategies in integrated pest management. *Annual Review of Entomology*, **52**, 375–400.

Cossé, A.A., Bartelt, R.J., Weaver, D.K. & Zilkowski B.W. (2002) Pheromone components of the wheat stem sawfly: identification, electrophysiology, and field bioassay. *Journal of Chemical Ecology*, **28**, 407–423.

Daisy, B.H., Strobel, G.A., Castillo, U., Ezra, D., Sears, J., Weaver, D.K. & Runyon, J.B. (2002) Naphthalene, an insect repellent, is produced by *Muscodor vitigenus*, a novel endophytic fungus. *Microbiology*, **148**, 3737–3741.

Dudareva, N., Pichersky, E. & Gershenzon, J. (2004) Biochemistry of plant volatiles. *Plant Physiology*, **135**, 1893–1902.

Fäldt, J., Arimura, G-i., Gershenzon, J., Takabayashi, J. & Bohlmann, J. (2003) Functional identification of AtTPS03 as (*E*)-β-ocimene synthase: a monoterpene synthase catalyzing jasmonate- and wound-induced volatile formation in *Arabidopsis thaliana*. *Planta*, **216**, 745–751.

Foster, S.P. & Harris. M.O. (1997) Behavioural manipulation methods for insect pest management. *Annual Review of Entomology*, **42**, 123–146.

Gols, R., Roosjen, M., Dijkman, H. & Dicke, M. (2003) Induction of direct and indirect plant responses by jasmonic acid, low spider mite densities, or a combination of jasmonic acid treatment and spider mite infestation. *Journal of Chemical Ecology*, **29**, 2651–2666.

Gouinguené, S., Degen, T. & Turlings, T.C.J. (2001). Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecology*, **11**, 9–16.

Hamilton-Kemp, T.R. & Anderson, R.A. (1984) Volatile compounds from *Triticum aestivum*. *Phytochemistry*, **23**, 1176–1177.

Hamilton-Kemp, T.R. & Anderson, R.A. (1986) Volatiles from winter wheat: identification of additional compounds and effects of tissue source. *Phytochemistry*, **25**, 241–243.

Hatanaka, A. (1993) The biogeneration of green odour by green leaves. *Phytochemistry*, **34**, 1201–1218.

Holmes, N.D. (1977) The effect of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), on the yield and quality of wheat. *Canadian Entomologist*, **109**, 1591–1598.

Holmes, N.D. & Peterson, L.K. (1960) The influence of the host on oviposition by the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). *Canadian Journal of Plant Science*, **40**, 29–46.

Kappers, I.F., Aharoni, A., Herpen van, T.W.J.M., Luckerhoff, L.L.P., Dicke, M., Bouwmeester, H.J. (2005) Genetic engineering of terpenoid metabolism attracts bodyguards to *Arabidopsis*. *Science*, **309**, 2070–2072.

Katsoyannos, B.I. & Guerin, P.M. (1984) Hexanol: a potent attractant for the black fig fly, *Silba adipata*. *Entomologia Experimentalis et Applicata*, **35**, 71–74.

Khan, Z.R. & Pickett, J.A. (2004) The 'push-pull' strategy for stemborer management: a case study in exploiting biodiversity and chemical ecology. *Ecological Engineering for Pest Management: Advances in Habitat Manipulation for Arthropods* (ed. by G. M. Gurr, S. D. Wratten, and M. A. Altieri), pp. 155–164. CSIRO, Australia.

Khan, Z.R., Midega, C.A.O., Hassanali, A., Pickett, J.A., Wadhams L.J. & Wanjoya, A. (2006) Management of witchweed, *Striga hermonthica*, and stemborers in sorghum, *Sorghum bicolor*, through intercropping with greenleaf desmodium, *Desmodium intortum*. *International Journal of Pest Management*, **52**, 297–302.

Leskey, T.C., Zhang, A. & Herzog, M. (2005) Nonfruiting host tree volatile blends: novel attractants for the plum curculio (Coleoptera: Curculionidae). *Environmental Entomology*, **34**, 785–793.

Martel, J.W., Alford, A.R. & Dickens, J.C. (2005a) Synthetic host volatiles increase efficacy of trap cropping for management of

Colorado potato beetle, *Leptinotarsa decemlineata* (Say). *Agricultural and Forest Entomology*, **7**, 79–86.

Martel, J.W., Alford, A.R., Dickens, J.C. (2005b) Laboratory and greenhouse evaluation of a synthetic host volatile attractant for Colorado potato beetle, *Leptinotarsa decemlineata* (Say). *Agricultural and Forest Entomology*, **7**, 71–78.

Miller, J.R. & Cowles, R.S. (1990) Stimulo-deterrent diversion: a concept and its possible application to onion maggot control. *Journal of Chemical Ecology*, **16**, 3197–3212.

Morrill, W.L. & Kushnak, G.D. (1999) Planting date influence on the wheat stem sawfly (Hymenoptera: Cephidae) in spring wheat. *Journal of Agricultural and Urban Entomology*, **16**, 123–128.

Morrill, W.L., Gabor, J.W. & Kushnak, G.D. (1992) Wheat stem sawfly (Hymenoptera: Cephidae) damage and detection. *Journal of Economic Entomology*, **85**, 2413–2417.

Morrill, W.M., Gabor, J.W., Weaver, D.K., Kushnak, G.D. & Irish, N.J. (2000) Effect of host plant quality on the sex ratio and fitness of female wheat stem sawflies (Hymenoptera: Cephidae). *Environmental Entomology*, **29**, 195–199.

Nakamuta, K., Leal, W.S., Nakashima, T., Tokoro, M., Ono, M. & Nakanishi, M. (1997) Increase of trap catches by a combination of male sex pheromones and a floral attractant in longhorn beetle, *Anaglyptus subfasciatus*. *Journal of Chemical Ecology*, **23**, 1635–1639.

Nakashima, Y., Birkett, M.A., Pye, B.J., Pickett, J.A. & Powell W. (2004) The role of semiochemicals in the avoidance of the seven-spot labybird *Coccinella septempunctata*, by the aphid parasitoid, *Aphidius ervi*. *Journal of Chemical Ecology*, **30**, 1103–1116.

Nansen, C., Weaver, D.K., Sing, S.E., Runyon, J.B., Morrill, W.L., Grieshop, M.J., Shannon, C.L. & Johnson, M.L. (2005) Within-field spatial distribution of *Cephus cinctus* (Hymenoptera: Cephidae) larvae in Montana wheat fields. *Canadian Entomologist*, **137**, 202–214.

Niinemets, U., Loreto, F. & Reichstein, M. (2004) Physiological and physico-chemical controls on foliar volatile organic compound emissions. *Trends in Plant Science*, **9**, 180–186.

Oehlschlager, A.C., Chinchilla, C., Castillo, G. & Gonzalez, L. (2002) Control of red ring disease by mass trapping of *Rhynchophorus palmarum* (*Coleoptera: Curculionidae*). *Florida Entomologist*, **85**, 507–513.

Pedigo, L.P. (2002) *Entomology and Pest Management*, 4th edn. Prentice Hall, Upper Saddle River, New Jersey.

Pettersson, J., Pickett, J.A., Pye, B.J., Quiroz, A., Smart, L.E., Wadhams, L.J. & Woodcock, C.M. (1994) Winter host component reduces colonization by bird-cherry-oat aphid, *Rhopalosiphum padi* (l.) (Homoptera, Aphididae), and other aphids in cereal fields. *Journal of Chemical Ecology*, **20**, 2565–2574.

Pickett, J.A., Wadhams, L.J. & Woodcock, C.M. (1997) Developing sustainable pest control from chemical ecology. *Agriculture Ecosystems and Environment*, **64**, 149–156.

Pickett, J.A., Rasmussen, H.B., Woodcock, C.M., Matthes, W. & Napier, J.A. (2003) Plant stress signaling: understanding and exploiting plant-plant interactions. *Biochemical Society Transactions*, **31**, 123–127.

Piesik, D., Weaver, D.K., Peck, G.E. & Morrill, W.L. (2006) Mechanically injured wheat plants release greater amounts of linalool and linalool oxide. *Journal of Plant Protection Research*, **46**, 29–39.

Quiroz, A. & Niemeyer, H.M. (1998) Olfactometer-assessed responses of aphid *Rhopalosiphum padi* to wheat and oat volatiles. *Journal of Chemical Ecology*, **24**, 113–124.

Quiroz, A., Pettersson, J., Pickett, J.A., Wadhams, L.J. & Niemeyer, H.M. (1997) Semiochemicals mediating spacing behaviour of bird cherry-oat aphid, *Rhopalosiphum padi*, feeding on cereals. *Journal of Chemical Ecology*, **23**, 2599–2607.

Reddy, A., Gadi, V.P. & Guerrero, A. (2004) Interactions of insect pheromones and plant semiochemicals. *Trends Plant Science*, **9**, 253–261.

Runyon, J.B., Morrill, W.L., Weaver, D.K. & Miller, P.R. (2002) Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) by *Bracon cephi* and *B. lissogaster* (Hymenoptera: Braconidae) in wheat fields bordering tilled and untilled fallow in Montana. *Journal of Economic Entomology*, **95**, 1130–1134.

Runyon, J.B., Mescher, M.C. & De Moraes, C.M. (2006) Volatile chemical cues guide host location and host selection by parasitic plants. *Science*, **313**, 1964–1967.

Ruther, J. & Mayer, C.J. (2005) Response of garden chafer, *Phyllopertha horticola*, to plant volatiles: from screening to application. *Entomologia Experimentalis et Applicata*, **115**, 51–59.

Ruther, J. & Tolasch, T. (2004) Attraction of garden chafer, *Phyllopertha horticola*, to floral Japanese beetle lure. *Journal of Applied Entomology*, **128**, 158–160.

SAS (1998) *SAS/STAT User's Guide*, Version 6, 4th edn. SAS Institute, Cary, North Carolina.

Shelton, A.M. & Badenes-Perez, F.R. (2006) Concepts and application of trap cropping in pest management. *Annual Review of Entomology*, **51**, 285–308.

Smith, C.M. (1989) *Plant Resistance to Insects*. Wiley, New York, New York.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*, 3rd edn. W. H. Freeman and Co., New York, New York.

Turlings, T.C.J. & Benrey, B. (1998) Effects of plant metabolites on the behaviour and development of parasitic wasps. *Ecoscience*, **5**, 321–333.

Turlings, T.C.J. & Wäckers, F. (2004) Recruitment of predators and parasitoids by herbivore-injured plants. *Advances in Insect Chemical Ecology* (ed. by R.T. Cardé and J.G. Millar), pp. 21–75. Cambridge University Press, Massachusetts.

Turlings, T.C.J., Tumlinson, J.H., Eller, F.J. & Lewis, W.J. (1991) Larval-damaged plants: source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the microhabitat of its hosts. *Entomologia Experimentalis et Applicata*, **58**, 75–82.

Turlings, T.C.J., McCall, P.J., Alborn, H.T. & Tumlinson, J.H. (1993) An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *Journal of Chemical Ecology*, **19**, 411–425.

Turlings, T.C.J., Alborn, H.T., Loughrin, J.H. & Tumlinson, J.H. (2000) Volicitin, an elicitor of maize volatiles in oral secretions of *Spodoptera exigua*: isolation and bioactivity. *Journal of Chemical Ecology*, **26**, 189–202.

Weaver, D.K., Sing, S.E., Runyon, J.B. & Morrill, W.L. (2004) Potential impact of practices on wheat stem sawfly (Hymenoptera: Cephidae) and associated parasitoids. *Journal of Agricultural and Urban Entomology*, **21**, 271–287.

Weaver, D.K., Nansen, C., Runyon, J.B., Sing, S.E. & Morrill, W.L. (2005) Spatial distributions of *Cephus cinctus* Norton (Hymenoptera: Cephidae) and its braconid parasitoids in Montana wheat fields. *Biological Control*, **34**, 1–11.

Weiss, M.J., Morrill, L.W. & Reitz, L.L. (1992) Wheat stem sawfly (Hymenoptera: Cephidae) revisited. *American Entomologist*, **38**, 241–245.

Zadoks, J.C., Chang, T.T. & Konsak, C.F. (1974) A decimal code for growth stages of cereals. *Weed Research*, **14**, 15–21.

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