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Host selection by the wheat stem sawfly in winter wheat and the role of semiochemicals mediating oviposition preference

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Abstract

The wheat stem sawfly (WSS), *Cephus cinctus* Norton (Hymenoptera: Cephidae), causes significant damage in cereal crops in the northern Great Plains of North America. This study assessed oviposition preference in winter wheat, *Triticum aestivum* L. (Poaceae), and investigated how it is affected by the emission of semiochemicals, with the overall goal of enhancing trap crop efficacy. We studied five winter wheat cultivars that could be recommended as trap crops for WSS and compared them with regards to agronomic characteristics influencing oviposition behavior and their emission of behaviorally active volatiles. Subsequently, we evaluated oviposition preference on three selected cultivars, 'Norstar', 'Neeley', and 'Rampart', using choice tests at two plant growth stages. Most eggs were found in Norstar at both stages tested when females were exposed to the three cultivars simultaneously making it the preferred choice for a trap crop. Norstar also emitted more behaviorally active volatiles, primarily (*E*)- and (*Z*)-β-ocimene. The results for the effect of main stem height or diameter on oviposition was inconsistent between infested vs. uninfested stems within cultivars, although there was a correlation between infestation and height for younger plants. These results show that these agronomic characteristics, typically viewed as explanatory, did not clearly explain oviposition preference and suggest a role of β-ocimene in determining suitability for oviposition among these cultivars. This study supports previous findings suggesting oviposition preference in winter wheat involves several cues, including stem height and volatile attractants that may be important in determining suitability.

Introduction

The wheat stem sawfly (WSS), *Cephus cinctus* Norton (Hymenoptera: Cephidae), causes significant damage in cereal crops in the northern Great Plains of North America where infestations in both winter and spring wheat have approached 100% in some fields (Morrill et al., 1994). Crop damage is caused by larval boring in stems (Morrill et al., 1994) and annual losses have been estimated at over US $350 million per year in the United States of America and Canada (Beres et al., 2011a). As an example of the severity of the problem, of approximately 2 million hectares of wheat, *Triticum aestivum* L. (Poaceae), planted in Montana in 2010 (37% was winter wheat with the remainder planted in the spring), 34% of the area had the solid-stem trait (USDA NASS, 2010). Solid-stem wheat is grown only when losses can be mitigated by its resistance to lodging that is due to stem cutting by WSS larvae (Beres et al., 2011a,b). In addition, pesticide use is very infrequent due to its minimal effectiveness against stem-boring sawflies (Shanower & Hoelmer, 2004; Knodel et al., 2009). Thus, current control measures rely mainly on solid-stem wheat cultivars (Weiss & Morrill, 1992), which vary in their efficacy depending on cultural practices and environmental conditions (Farstad, 1940; Platt & Farstad, 1946; Miller et al., 1993). These limitations indicate that alternative management practices are needed (Beres et al., 2011a,b). Strategies such as trap cropping and management based on semiochemicals have shown promise as methods of

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crop protection for WSS (Morrill et al., 2001; Weaver et al., 2004) but additional research is required for their field application (Piesik et al., 2008; Weaver et al., 2009; Buteler et al., 2010). Wheat stem sawfly infestations create distinct edge effects (Nansen et al., 2005) and previous research by Morrill et al. (2001) reported a reduction in WSS infestation from a perimeter winter wheat trap crop on a field of spring wheat.

Trap crop effectiveness can be greatly improved by the use of semiochemicals that influence insect behavior (Cook et al., 2007). Considerable attention is being focused now on identification of volatiles in crop plants and the potential use of semiochemicals in the control of insect pests (Pickett et al., 1997, 2006; Agelopoulos et al., 1999; Witzgall et al., 2010). Moreover, it has long been recognized that detailed knowledge of the quantitative and qualitative properties of kairomones in plant species and cultivars is essential to define their roles in insect attraction and oviposition in a scientifically designed trap crop system (Hokkanen, 1991). Recent studies have suggested that release of volatile compounds from host plants provides cues for female WSS that may aid in the determination of stems suitable for oviposition (Piesik et al., 2008; Weaver et al., 2009). Several host plant volatiles with behavioral activity have been identified in spring wheat: the green leaf volatiles (*Z*)-3-hexenyl acetate and (*Z*)-3-hexenol, the terpene β-ocimene, and 6-methyl-5-hepten-2-one (Piesik et al., 2008). A marked preference for a spring wheat cultivar that emits more (*Z*)-3-hexenyl acetate has been observed in greenhouse and field choice tests (Weaver et al., 2009). Oviposition preference in WSS is also influenced by height, developmental stage, and stem diameter. Quantitative trait loci for WSS oviposition preference have been identified (Sherman et al., 2010).

Adult WSS females are constrained to hosts which they can utilize with their saw-like ovipositor and wheat plants are vulnerable from stem elongation through anthesis. Spring and winter wheat are considered two flowering types of wheat, differing primarily in the 'VRN' gene which controls the ability to vernalize, defined as 'the acquisition or acceleration of the ability to flower via a chilling period' (Fu et al., 2005). Because of this trait, spring and winter wheat differ considerably in their growth habit and development, which translates into a marked difference in plant phenology, physiology, and germplasm (Crofts, 1989). In winter wheat, which is almost exclusively sown in the fall, the vegetative phase is prolonged given that it requires temperatures between 0 and 7 °C for 30–60 days for floral induction, generating a higher number of leaves in the main shoot (Acevedo et al., 2002). Winter wheat is more mature at the time of the WSS flight period so it is consistently exposed to infestation, i.e., longer than spring wheat, and therefore is more vulnerable to WSS. Testing the relationship between oviposition preference and agronomic characteristics in winter wheat could provide further understanding of the factors influencing WSS behavior. This is especially interesting considering that most studies have been conducted in spring wheat, and that WSS have adapted to utilize winter wheat in the northern Great Plains more recently than spring wheat (Morrill & Kushnak, 1996). Studying preference among winter wheat cultivars is particularly relevant because it can be an effective trap crop (Morrill et al., 2001).

A study by Buteler et al. (2010) evaluated the initial suitability of nine winter wheat cultivars adapted for cultivation in Montana as trap crops. In this study, cultivars were screened based on the phenological characteristics that influence WSS oviposition behavior that are described above, the emission of the attractant (*Z*)-3-hexenyl acetate (Weaver et al., 2009), and their agronomic performance in areas where infestations occur. Greater damage in field nurseries was observed in cultivars emitting more (*Z*)-3-hexenyl acetate (Buteler et al., 2010). These results suggest that there is potential to enhance the efficacy of trap crops to manage WSS by selecting the most attractive cultivars among those currently grown in the areas where WSS is a key pest.

Use of cultivars that are currently grown in the region of cultivation may lead to a greater adoption of trap cropping as a management practice (Weaver et al., 2009). The present study evaluates the attractiveness of winter wheat cultivars under controlled conditions and specifically addresses how this relates to the emission of behaviorally active volatiles, among other agronomic traits that affect oviposition behavior. We initially measured the emission of known behaviorally active volatile compounds from a larger pool of five cultivars with potential as trap crops, selected from Buteler et al. (2010), to manage WSS in wheat-fallow cropping. Subsequently, oviposition preference in greenhouse choice tests was evaluated for the three cultivars that exhibited the greatest potential as trap crops based on semiochemical emission, suitable phenological characteristics, and agronomic preference among growers.

Materials and methods

Insects

Adult WSS were reared from wheat stubble containing larvae in diapause, collected in a field with a history of sawfly infestation in Conrad Montana (48°8.00′N, 112°6.68′W). Temperature was maintained at 0–4 °C for 3–6 months to facilitate completion of the obligate larval diapause. After this, the material was placed in plastic Tupperware® boxes (Tupperware Corporation; Orlando, FL, USA) (70 × 35 × 20 cm) and held at room temperature (22–27 °C), until the adults emerged 4–5 weeks later. The boxes were opened daily and the emerged adults were held in glass 2-l Ball® Mason jars (Distributed by Hearthmark, LLC via Jarden Home Brands, Daleville, IN, USA) until they were used in experiments. The glass jars contained moistened filter paper and a solution of water and cane sugar [10% (wt/vol) sucrose solution]. To minimize host deprivation time, all bioassays were conducted with adults within 24 h of eclosion, and usually only a few hours after this event. These conditions mimic those preferred in nature, where typically adults start laying eggs shortly after emergence but are most active around midday (Ainslie, 1929).

Plants

Five winter wheat cultivars meeting criteria for potential trap crops for WSS in Montana (Buteler et al., 2010) were studied. The criteria included plant height, winter hardiness, popularity among growers, presence of the sawfly resistance trait, developmental rate, and emission of the attractant (*Z*)-3-hexenyl acetate. These cultivars were Norstar (CItr 17735) (Grant, 1980), BigSky (PI 619166) (Bruckner et al., 2003), Morgan (USDA-AMS, PVPO, 1997) (PI 599336), Rampart (PI 593889) (Bruckner et al., 1997), and Neeley (CItr 17860) (Sunderman, 1983).

Experiments were performed under controlled conditions at the Plant Growth Center, Montana State University (MSU-PGC, Bozeman, MT, USA) as previously described in Piesik et al. (2008). The seeds were planted singly in 2.5 × 17 cm plastic cones, and placed in a greenhouse with supplemental light (GE Multi-Vapor Lamps-model MVR1000/C/U, GE Lighting; General Electric, Cleveland, OH, USA), controlled temperature, and ambient humidity (typically ranging from 20 to 40%). The photoperiod was L15:D9, daytime temperature was 22 ± 2 °C, and overnight temperature was 20 ± 2 °C. The winter wheat plants were vernalized for 8 weeks at 4 ± 1 °C when they achieved a developmental stage of Zadoks 13 (three unfolded leaves), using the decimal stage developed by Zadoks et al. (1974). After vernalization, the plants were transplanted into tapered square pots (13 × 13 × 13.5 cm) at a density of three plants per pot and grown in the greenhouse until used experimentally. The soil consisted of equal parts of MSU-PGC soil mix (equal parts of sterilized Bozeman silt loam soil and washed concrete sand with Canadian sphagnum peat moss incorporated) and Sunshine Mix #1 (Canadian sphagnum peat moss, perlite, vermiculite, and Dolmitic lime; Sun Gro Horticulture, Bellevue, WA, USA) (Piesik et al., 2006).

Plants used for experimentation were at a developmental stage of Zadoks 33, when 2–3 nodes are visible, or at Zadoks 49, which is the 'boot' stage prior to head emergence (Zadoks et al., 1974). These two stages were chosen because they approximate the extremes in the range of developmental stages preferred for oviposition according to Holmes & Peterson (1960).

Volatile collection and analysis

To quantify volatile compounds, intact wheat plants were placed in collection chambers in a volatile collection system (VCS) as previously described by Piesik et al. (2006). Wheat volatiles were collected by pulling air through traps for 8 h, between 10:00 and 18:00 hours (under an ambient, supplemented photophase). The main stem on each plant was enclosed in a glass volatile collection chamber (40 mm diameter × 800 mm long) that was attached to a volatile collection port and was open on the other end to enclose the plant. Glass filters (6.35 mm outer diameter × 76 mm long; Analytical Research Systems, Gainesville, FL, USA) containing 30 mg of Super-Q adsorbent (Alltech Associates, Deerfield, IL, USA) were inserted into each volatile collector port. Purified, humidified air was delivered at a rate of 1.0 l min^{-1} over the stem, and the flow and pressure were maintained by a regulated vacuum pump. A Teflon® sleeve (provided by Analytical Research Services, Inc. Gainesville, FL, USA) encircled the base of the stem and was taped to the glass VCS tube to prevent surrounding greenhouse air from entering the system.

Traps were eluted with 200 µl of hexane and transferred to a glass insert held in a 1.5-ml crimp-top glass vial. After elution, 7.3 ng of the internal standard, (*E*)-2-nonene (Sigma-Aldrich Chemical, Milwaukee, WI, USA) in hexane, was added. Samples were analyzed by gas chromatography (Agilent 6890 instrument; Agilent Technologies, Santa Clara, CA, USA) coupled to a mass selective detector (MSD, Agilent 5973 instrument; Agilent Technologies) according to Piesik et al. (2008). Samples were analyzed for (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, (*E*)- and (*Z*)-β-ocimene, and 6-methyl-5-hepten-2-one quantities and the results are presented as ng g^{-1} h^{-1} or as ng h^{-1}. Quantitation was completed using single point response factors for each compound that were developed using the MSD (Hites, 1997).

Identities of volatile compounds were determined by comparison of the mass spectrum to those within the NIST mass spectral library (Rev. D.02.00) and by comparison to retention times for authentic standards. The (*E*)-β-ocimene was synthesized in the Biological Chemistry Department at Rothamsted Research (Hertfordshire, UK), as described by Birkett et al. (2006). (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, (*Z*)-β-ocimene, and 6-methyl-5-hepten-2-one were obtained from Sigma-Aldrich (St. Louis, MI, USA). All peaks were examined for purity while verifying their identity, and peaks that were not able to be reliably quantified were not included in the analysis, which happened occasionally for the known attractant (*Z*)-3-hexen-1-ol. Each experiment consisted of two plants of each of the five cultivars randomly distributed within the VCS, whereas a control consisted of the airspace above a pot containing soil only. The experiment was replicated three times with plants in Zadoks 33, and five times with plants in Zadoks 49.

Preference tests

Based on the results from the volatile collections, and other agronomic characteristics, we subsequently compared attractiveness of three winter wheat cultivars to WSS in choice and no-choice tests. The three cultivars tested were Norstar, Rampart, and Neeley. Rampart was selected because it has been previously reported to reduce WSS infestations as a trap crop (Morrill et al., 2001) and it is a lodging resistant, solid-stem cultivar. Norstar and Neeley were selected because they are among the tallest cultivars and remain suitable for oviposition longer because of their slower development (Buteler et al., 2010). It should be noted that commercial quantities of Norstar are currently limited, but reserves will remain readily obtainable because it is a key genetic source of winter hardiness for development of new cultivars. Thus, a larger supply could easily become available for perimeter trap cropping if it proved to be a better trap than other cultivars.

To investigate discrimination among the cultivars, Norstar, Neeley, and Rampart were simultaneously presented in three cages to groups of 10 females. Choice tests were conducted inside 91.4 × 66.7 × 91.4 cm screen cages with 530 μm mesh openings (BioQuip Products, Rancho Dominguez, CA, USA). Three cages were placed in the greenhouse and each cage contained a single plant of each cultivar in square tapered pots (13 × 13 × 13.5 cm), evenly spaced within the cage. The corner of the cage in which each cultivar was placed was randomized. The females were released in the center of each of the cages, with five males to facilitate mating and were allowed to oviposit for 2 days. The pots were then removed from the cages and stems were dissected to count the eggs. Above-ground plant biomass, stem diameter, and stem height were recorded. Stem diameter was measured using an electronic caliper to the nearest 0.01 mm. The experiment was repeated five times with plants in the early developmental stage (Zadoks 33) and five times with plants at the later developmental stage (Zadoks 49). For the no-choice tests, individual plants of each cultivar were simultaneously enclosed in plastic tubes (4.5 cm diameter × 62 cm tall) and three female sawflies and one male were released in each tube. The three enclosed plants were placed next to each other in the greenhouse, so that all of them were in similar microenvironments. After 2 days, tubes were removed and the plants were dissected to count the eggs. Three no-choice replicates were conducted with plants at Zadoks 33 and another three with plants at Zadoks 49.

Statistical analysis

The volatile collection data were subjected to analysis of variance (ANOVA) (Proc MIXED; SAS Institute, 1998) to determine differences between total amounts of behaviorally active compounds emitted by the cultivars at the two developmental stages. Cultivar and plant stage were included as the fixed effects and plant within variety and replicate of the experiment were included as random factors. The autoregressive correlation structure was fitted to the model. A factorial multivariate analysis of variance (MANOVA) (Proc GLM) was also conducted. The Wilks' Lambda test statistic was used to discriminate significant main effects in the MANOVA. Univariate ANOVAs were conducted to further investigate potential differences between the cultivars for each compound. The data were square-root transformed to better meet the assumption of normality of the distribution and are presented as means of the untransformed data.

Results from no-choice tests were subjected to ANOVA to compare the number of eggs per plant and the proportion of infested stems among cultivars, with cultivar as fixed effect and cage and replicate of experiment as random effects (Proc MIXED). Each developmental stage was analyzed separately.

In choice tests, differences in number of eggs per plant among cultivars were analyzed using ANOVA with cultivar, developmental stage, and date of experiment as fixed effects, and cage as a random effect (Proc MIXED). To account for multiple tillers in a plant and given that cultivars may vary in their number of infestable tillers we also analyzed the number of eggs per stem. Differences in number of eggs per stem among cultivars were analyzed using ANOVA with cultivar and stage as fixed effects and cage or plant and replicate of experiment as random effects (Proc MIXED).

In all cases, significantly different least square means ($P<0.05$) were separated using the Tukey adjusted option in SAS (SAS Institute, 1998). The variables eggs per stem and eggs per plant were transformed using $\sqrt{(x + 0.5)}$ before analysis to stabilize normality. Stem height was transformed using $\log (x + 0.5)$ and stem diameter was transformed by $\ln(x)$. Data are presented as means of the untransformed data.

Differences in mean height and stem diameter for infested and uninfested stems were compared using t-tests

(Proc TTEST). Also, to assess relationships between stem height, stem diameter, and number of eggs, we performed stepwise multiple regression analysis (Proc REG). Differences in plant weight, height, and stem diameter, which could also influence oviposition preference between the cultivars, were analyzed by ANOVA.

Results

Behaviorally active volatiles emitted

Differences in plant biomass between cultivars ($F_{4,66}$ = 3.65, P<0.01), and stages ($F_{1,66}$ = 267.3 P<0.0001) were observed. Therefore, amounts were analyzed as both ng h^{-1} (entire stem) and as ng g^{-1} h^{-1} (biomass) to understand whether differences in volatiles among cultivars may be related to differences in physiology or biomass (Buteler et al., 2009).

There were differences in the total amounts of behaviorally active volatiles emitted by cultivars (when analyzed as the entire stem as well as when correcting for biomass $F_{4,68}$ = 7.23, P<0.0001; entire stems $F_{4,68}$ = 10.51, P< 0.0001) as well as a difference between stages (biomass $F_{1,68}$ = 6.33, P<0.01; entire stems $F_{1,68}$ = 12.40, P<0.001). Overall, norstar emitted the greatest amount of behaviorally active volatiles (Table 1). Subsequent multivariate analysis (MANOVA) comparing amounts of each of the behaviorally active volatiles indicated that there was an overall difference in the amount of volatile compounds emitted by different cultivars (biomass Wilks' Lambda = 0.52, $F_{16,202.27}$ = 3.04, P<0.0001; entire stem Wilks' Lambda = 0.41, $F_{16,205.33}$ = 4.28, P<0.001). There was also a difference between developmental stages in the total amount of compounds emitted (biomass Wilks' Lambda = 0.44, $F_{4,66}$ = 21.33, P<0.0001; entire stem Wilks' Lambda = 0.72, $F_{4,67}$ = 6.35, P<0.001). The amount of (*E*)-β-ocimene emitted by entire stems was greater at the later developmental stage of Zadoks 49 and the amount of (*Z*)-3-hexenyl acetate was greater at the earlier developmental stage of Zadoks 33 (P<0.05). The amounts of 6-methyl-5-hepten-2-one and (*Z*)-3-hexenyl acetate emitted were greater at the earlier developmental stage when corrected for biomass (P<0.05).

The two β-ocimene isomers averaged 53% at Zadoks 33 and 77% at Zadoks 49, of the total behaviorally active volatiles emitted and were the only compounds that differed among cultivars. The amount of (*E*)-β-ocimene corrected for biomass ($F_{4,65}$ = 11.45, P<0.0001) and for the entire stems ($F_{4,65}$ = 12.76, P<0.0001) plus (*Z*)-β-ocimene corrected for biomass ($F_{4,65}$ = 2.20, P = 0.08) and for the entire stems ($F_{4,65}$ = 4.73, P<0.01) were all different among the cultivars (Table 2). In general, plants emitted more (*E*)-β-ocimene at Zadoks 49. Norstar emitted more (*E*)-β-ocimene than BigSky, Morgan, Rampart, and Neeley (corrected for biomass and for entire stem: P<0.05), and more (*Z*)-β-ocimene than Rampart and Neeley (corrected for biomass and for entire stem; P<0.1). For (*Z*)-3-hexenyl acetate and 6-methyl-5-hepten-2-one, no differences among cultivars were observed (P>0.1). Winter wheat plants emitted on average 0.83 ± 0.10 and 1.02 ± 0.16 ng h^{-1} of 6-methyl-5-hepten-2-one at Zadoks 33 and 49, respectively. The overall mean of (*Z*)-3-hexenyl acetate for winter wheat plants was 3.51 ± 0.68 and 1.65 ± 0.26 ng h^{-1} at Zadoks 33 and 49, respectively. The amount of the reported attractant (*Z*)-3-hexen-1-ol emitted by spring wheat plants (Piesik et al., 2008) was not analyzed because the peaks were frequently present only in trace amounts.

Table 1 Mean amount (± SE) of total behaviorally active volatile compounds emitted by five winter wheat cultivars at two developmental stages (Zadoks 33 and 49), expressed for entire stems and corrected for stem biomass

		Amount	
Cultivar	Zadoks stage	Entire stem (ng h^{-1})	Biomass corrected (ng g^{-1} h^{-1})
Bigsky	33	9.03 ± 3.81bc	1.04 ± 0.27ab
	49	14.40 ± 3.81ab	0.73 ± 0.19ab
Morgan	33	9.36 ± 5.76bc	1.21 ± 0.59ab
	49	11.78 ± 1.63ab	0.59 ± 0.09ab
Neeley	33	9.91 ± 2.42bc	1.46 ± 0.25a
	49	9.06 ± 3.46bc	0.52 ± 0.21b
Norstar	33	14.86 ± 3.66ab	1.93 ± 0.47a
	49	30.05 ± 7.56a	1.39 ± 0.42a
Rampart	33	3.98 ± 0.80c	0.66 ± 0.13ab
	49	5.39 ± 1.15bc	0.33 ± 0.7b

Means within a column followed by different letters are significantly different (ANOVA followed by Tukey pairwise comparison: P<0.05).

Preference tests

In choice tests, the number of eggs per plant differed among cultivars ($F_{2,533}$ = 8.1, P<0.001), and stages ($F_{1,533}$ = 49.34, P<0.0001) without significant interactions among these factors. Norstar received more eggs than Neeley and Rampart (Table 3). The winter wheat cultivars differed in mean plant weight ($F_{2,28}$ = 4.36, P<0.05) and mean stem height ($F_{2,222}$ = 4.24, P<0.05), but not in their stem diameter ($F_{2,221}$ = 1.19, P>0.1) at Zadoks 49 (Table 3). Biomass and stem height were greatest for Norstar (P<0.05) at this stage. At Zadoks 33, the cultivars did not differ in biomass ($F_{2,28}$ = 1.18, P>0.1), mean stem height ($F_{2,290}$ = 2.07, P>0.1), or stem diameter ($F_{2,290}$ = 1.65, P>0.1) (Table 3). We also investigated the relation-

Table 2 Mean amount (± SE) of the behaviorally active volatile compound β-ocimene emitted by five winter wheat cultivars at two developmental stages (Zadoks 33 and 49), expressed for entire stems and corrected for stem biomass

		Compound			
		(*E*)-β-ocimene		(*Z*)-β-ocimene	
Cultivar	Zadoks stage	Entire Stem (ng h^{-1})	Biomass corrected (ng g^{-1} h^{-1})	Entire stem (ng h^{-1})	Biomass corrected (ng g^{-1} h^{-1})
Bigsky	33	3.87 ± 1.85bcd	0.44 ± 0.14bc	0.32 ± 0.19ab	0.045 ± 0.03ab
	49	11.31 ± 3.43b	0.57 ± 0.17abc	0.34 ± 0.08ab	0.016 ± 0.003ab
Morgan	33	4.19 ± 2.93cd	0.52 ± 0.31bc	0.13 ± 0.05ab	0.027 ± 0.01ab
	49	9.35 ± 1.81b	0.48 ± 0.10abc	0.32 ± 0.06ab	0.017 ± 0.003ab
Neeley	33	5.42 ± 1.98bcd	0.77 ± 0.23abc	0.18 ± 0.11b	0.027 ± 0.02b
	49	5.42 ± 2.83bcd	0.30 ± 0.16c	0.26 ± 0.09ab	0.016 ± 0.006ab
Norstar	33	9.95 ± 2.99bc	1.26 ± 0.36ab	0.26 ± 0.05ab	0.034 ± 0.03ab
	49	26.79 ± 7.26a	1.24 ± 0.39a	0.67 ± 0.15a	0.029 ± 0.007a
Rampart	33	0.97 ± 0.19d	0.16 ± 0.03c	0.13 ± 0.11b	0.033 ± 0.03b
	49	2.68 ± 0.72bcd	0.16 ± 0.04c	0.09 ± 0.03b	0.007 ± 0.003b

Means within a column followed by different letters are significantly different (ANOVA followed by Tukey pairwise comparison: $P<0.05$).

Table 3 Mean (± SE) number of eggs per plant, and height, diameter, and biomass of stems of three winter wheat cultivars at two developmental stages (Zadoks 33 and 49)

	No. eggs		Height (cm)		Diameter (cm)		Mass (g)	
Cultivar	Zadoks 33	Zadoks 49	Zadoks 33	Zadoks 49	Zadoks 33	Zadoks 49	Zadoks 33	Zadoks 49
Rampart	0.39 ± 0.09a	1.12 ± 0.16a	20.12 ± 0.89a	33.08 ± 1.45ab	2.71 ± 0.06a	2.67 ± 0.08a	16.70 ± 2.16a	16.28 ± 1.37a
Neeley	0.32 ± 0.06a	1.22 ± 0.19a	19.99 ± 1.08a	29.04 ± 1.56a	2.93 ± 0.08a	2.83 ± 0.09a	22.18 ± 3.73a	16.65 ± 1.2a
Norstar	0.85 ± 0.19b	1.76 ± 0.19b	21.52 ± 0.93a	36.41 ± 1.86b	2.94 ± 0.07a	2.76 ± 0.09a	24.92 ± 4.80a	20.01 ± 1.63b

Means within a column followed by different letters are significantly different (ANOVA followed by Tukey pairwise comparison: $P<0.05$).

ship between numbers of eggs per stem and stem height, or stem diameter independently of cultivar. We sought to determine whether or not oviposition could be explained solely by these factors in winter wheat. Multiple linear regression analysis with stem diameter and height as explanatory variables indicated no significant relationship between stem height or stem diameter and number of eggs per stem ($R^2 = 0.03$, $F_{2,235} = 1.60$, $P>0.05$) with plants in Zadoks 49. At Zadoks 33, only stem height was significant in explaining part of the variability in number of eggs per stem, although the relationship was weak ($R^2 = 0.014$, height: $F_{1,305} = 4.17$, $P<0.05$).

Infested stems of Norstar tended to be taller than uninfested ones at both stages (Figure 1A) (Zadoks 33: $P<0.0001$; Zadoks 49: $P<0.05$). Infested stems of Neeley were shorter than uninfested stems at Zadoks 49 (Zadoks 33: $P>0.1$; Zadoks 49: $P<0.05$), whereas infested stems of Rampart were longer than uninfested stems at Zadoks 33 (Zadoks 33: $P<0.1$; Zadoks 49: $P>0.05$). Stem diameter differed between infested and uninfested stems only in Norstar at Zadoks 33 (Zadoks 33: $P<0.001$; Zadoks 49: $P>0.1$) and in Neeley at both stages (Zadoks 33: $P<0.01$; Zadoks 49: $P<0.05$) (Figure 1B). Infested Norstar stems were wider than uninfested stems (significant only at Zadoks 33), whereas infested Neeley stems were thinner than uninfested ones at Zadoks 49 and wider at Zadoks 33.

Based on these results, ANOVA was conducted to detect differences in oviposition preference among the three cultivars, with stem height as a covariate for plants at Zadoks 33. For plants in Zadoks 49 we observed differences among cultivars ($P<0.01$) and date of experiment ($P<0.0001$) but no interaction between cultivar and date of experiment ($P>0.1$) in the number of eggs per stem. The number of eggs per stem was greater in Norstar than in Neeley and Rampart (Figure 2). For plants in Zadoks 33, the analysis revealed that there was a difference in eggs per stem among

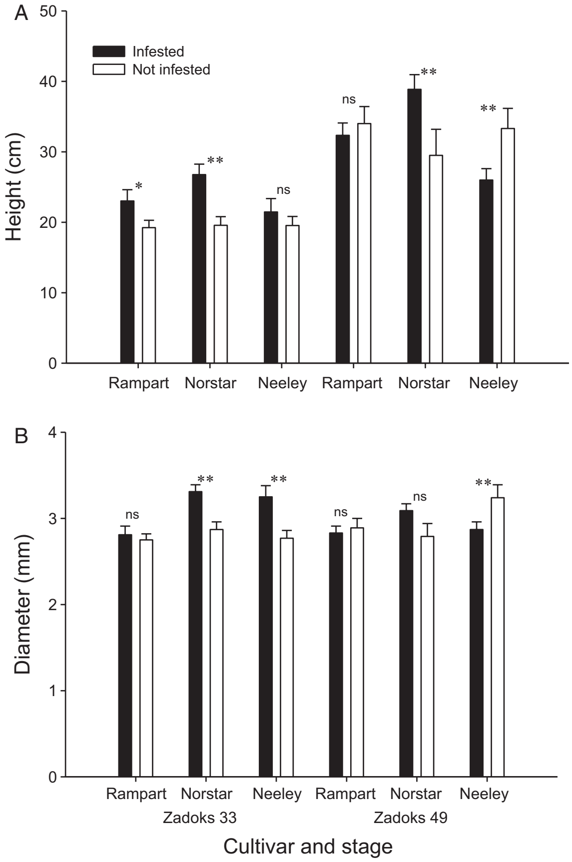

Figure 1 (A) Mean (+ SE) height and (B) diameter of infested and uninfested stems from three winter wheat cultivars at two developmental stages (Z33 = Zadoks 33, Z49 = Zadoks 49) suitable for infestation by the wheat stem sawfly. Comparisons are made between infestation status for each cultivar and stage. **Indicates significant difference at P<0.05; *indicates significant difference at P<0.1; ns indicates no significant difference (Student's t-test).

cultivars (P<0.01) as well as an effect of stem height on eggs per stem (P<0.0001). Taller Norstar stems tended to receive more eggs. In no-choice tests, there were no differences among cultivars in number of eggs or proportion of infested stems (Zadoks 33 and 49: all P>0.1) (Table 4).

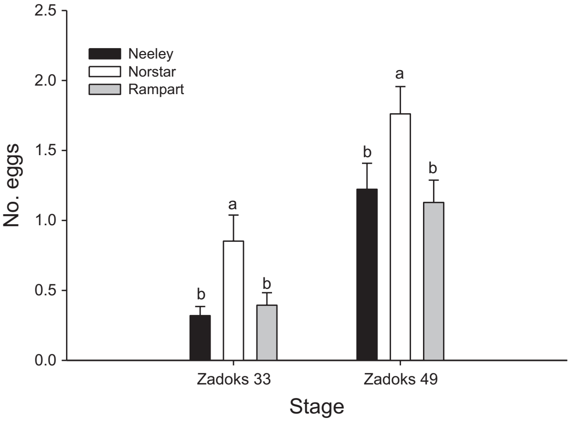

Figure 2 Mean (+ SE) number of eggs per stem in three winter wheat cultivars at two developmental stages (Zadoks 33 and 49) suitable for infestation by the wheat stem sawfly. Comparisons were done among cultivars within a stage. Bars with different letters within stages are significantly different (ANOVA followed by Tukey pairwise comparison: P<0.05).

Discussion

Results from the no-choice tests suggest that none of the cultivars tested were inherently less suitable than the others. However, when females were exposed to the three cultivars simultaneously they discriminated among cultivars and more eggs were counted in Norstar at both developmental stages tested. Results of this study also show that, in general, infested stems from younger plants (Zadoks 33) were taller than uninfested ones. This agrees with previous studies that suggest that oviposition in WSS is related to stem height, especially within the same stage (Seamans, 1928; Holmes & Peterson, 1960; Buteler et al., 2009, 2010). However, infested Neeley stems from older plants tended to be shorter than uninfested ones and no significant differences in height were observed between stems from the older infested and uninfested Rampart plants. There was no consistent relationship between infestation and stem diameter, although for plants at Zadoks 33, infested stems of Norstar and Neeley were wider than uninfested stems. We suggest that height plus stem diame-

Table 4 Mean (± SE) proportion of infested stems and number of eggs in no-choice tests with three winter wheat cultivars at two developmental stages (Zadoks 33 and 49)

	Zadoks 33		Zadoks 49	
Cultivar	Infested stems/plant	No. eggs/plant	Infested stems/plant	No. eggs/plant
Rampart	0.41 ± 0.10	0.72 ± 0.20	0.26 ± 0.09	1.67 ± 0.67
Neeley	0.44 ± 0.12	1.0 ± 0.49	0.28 ± 0.07	1.17 ± 0.31
Norstar	0.51 ± 0.14	0.93 ± 0.26	0.29 ± 0.11	1.17 ± 0.40

Means within a column were not significantly different (ANOVA followed by Tukey pairwise comparison: P>0.05).

ter could be limiting factors for oviposition when plants are young, because there are fewer internodes developed in the stem and consequently there is less surface area for the females to explore before oviposition (Buteler et al., 2009). Shorter, narrower stems could also be limiting for subsequent larval development inside the stem.

When plants are in more advanced stages, most stems available are likely to exceed a potential threshold of acceptable height. In that case, it is likely that other variables become more important in selecting stems for oviposition. Among other factors, semiochemical production in host plants plays a significant role in orientation and oviposition behavior in WSS (Piesik et al., 2008; Weaver et al., 2009). In our study, cultivar differences in oviposition preference could not be solely explained by the agronomical characteristics reported as WSS oviposition cues. Previous studies on the chemical ecology of WSS interactions with wheat have recorded differences in emission of the attractive compound (*Z*)-3-hexenyl acetate in spring wheat (Weaver et al., 2009) and winter wheat (Buteler et al., 2010) cultivars. Based on the work by Buteler et al. (2010), the present study used cultivars emitting the most (*Z*)-3-hexenyl acetate, and more detailed analyses of overall volatile emissions were conducted. We found that there was variation among those cultivars in another known behaviorally active compound: β-ocimene [both the (*Z*)- and (*E*)- isomers]. Piesik et al. (2008) reported a positive behavioral response to commercial β-ocimene (containing both isomers) in Y-tube olfactometer experiments at concentrations of 1 ng h^{-1}. Interestingly, WSS preferred to lay eggs in the winter wheat cultivar that emitted more of this attractive compound, suggesting that it may play a role in the further discrimination of oviposition sites by females. Other reports in the literature demonstrate that quantitative differences in volatile emissions within a species are very common in cultivated plants (Dicke, 1999; Degen et al., 2004; Lou et al., 2006).

Secondary metabolites ubiquitously produced by plants are the most prevalent mechanism mediating host recognition, and insects are capable of detecting differences in blends of compounds to guide them to suitable hosts (Bruce et al., 2005). Different ratios of the same compounds could be providing signals to WSS females to evaluate differences in suitability among stems, and this may also vary by the type of wheat, given the differences in amounts of behaviorally active compounds that were observed in winter wheat compared to those in spring wheat (Weaver et al., 2009). Additional research is required using electroantennographic detection (EAD) with headspace wheat volatiles on WSS antennae to identify other biologically active compounds. Behavioral studies with the WSS using blends of synthetic wheat volatiles would shed more light as to the role of each of these compounds on host selection. Results from such experiments could provide useful information for the development of a synthetic host plant derived attractant for WSS females. Additional studies solely evaluating the role of individual attractive compounds on oviposition are complicated by the inextricable interaction among all the plant characteristics involved. Studies with mutant plants that do not emit one or more of the behaviorally active compounds (Dudareva & Pichersky, 2008) could be used to test their importance in host attractiveness and suitability in this system.

The results from this study support previous findings (Piesik et al., 2008; Buteler et al., 2009, 2010) suggesting oviposition preference involves a complex set of host cues, of which olfactory cues might be an important factor in the decision-making process of selecting a stem for oviposition. Wheat stem sawfly oviposition behavior, as in other insects (Hattori, 1988; Nottingham, 1988), is a process involving different host location cues as well as host acceptance cues (Ramaswamy, 1988). Although we could not separate olfactory cues from other host factors influencing WSS behavior, our results suggest that odors interact with other host stimuli to shape insect behavior (Buteler et al., 2009, 2010).

Improvement of trap crop effectiveness is needed before this practice is widely adopted (Beres et al., 2009) and the results obtained in the present study indicate that the choice of trap cultivar can have an impact on WSS preference, leading to a concentration of feeding larvae in the trap. Thus, trap cropping of this pest may be improved by selecting a cultivar like Norstar, which emits more attractive semiochemicals and is favored by ovipositing females. Our results support the findings by Weaver et al. (2009) suggesting that the effectiveness of trap crops for this insect can be enhanced by taking advantage of the naturally-occurring plant semiochemicals (Khan & Pickett, 2004; Shelton & Badenes-Perez, 2006). Additional research evaluating the effectiveness of the cultivars evaluated as potential trap crops should be conducted in large-scale field trials. Additional studies on the effect of plant semiochemicals on host finding by WSS parasitoids could also aid in developing a more effective trap crop. Manipulation of plant volatiles by application of synthetic compounds and any effects on oviposition by WSS as well as its parasitoids could also be evaluated to test the potential of a semiochemically augmented trap crop to manage this pest.

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References

Acevedo E, Silva P & Silva H (2002) Wheat growth and physiology. Bread Wheat – Improvement and Production (ed. by BC Curtis, S Rajaram & H Gómez Macpherson), pp. 39–70. FAO Plant Production and Protection Series No. 30. FAO, Rome, Italy.

Agelopoulos N, Birkett MA, Hick AJ, Hooper AM, Pickett JA et al. (1999) Exploiting semiochemicals in insect control. Pesticide Science 55: 225–235.

Ainslie CN (1929) The western grass-stem sawfly – a pest of small grains. USDA Bulletin 157: 1–24.

Beres BL, Cárcamo HA & Bremer E (2009) Evaluation of alternative planting strategies to reduce wheat stem sawfly (Hymenoptera: Cephidae) damage to spring wheat in the Northern Great Plains. Journal of Economic Entomology 102: 2137–2145.

Beres BL, Cárcamo HA, Weaver DK, Dosdall LM, Evenden ML et al. (2011a) Integrating the building blocks of agronomy and biocontrol into an IPM strategy for wheat stem sawfly. Prairie Soils & Crops: Scientific Perspectives for Innovative Management 4: 54–65.

Beres BL, Dosdall LM, Weaver DK, Cárcamo HA & Spaner DM (2011b) Biology and integrated management of wheat stem sawfly and the need for continuing research. Canadian Entomologist 143: 105–125.

Birkett MA, Chamberlain K, Khan ZR, Pickett JA, Toshova T et al. (2006) Electrophysiological responses of the lepidopterous stemborers *Chilo partellus* and *Busseola fusca* to volatiles from wild and cultivated host plants. Journal of Chemical Ecology 32: 2475–2487.

Bruce TJA, Wadhams LJ & Woodcock CM (2005) Insect host location: a volatile situation. Trends in Plant Science 10: 269–274.

Bruckner PL, Kushnak GD, Berg JE, Wichman DM, Carlson GR et al. (1997) Registration of 'Rampart' wheat. Crop Science 37: 1004.

Bruckner PL, Berg JE, Carlson GR, Wichman DM, Riveland N et al. (2003) Registration of 'BigSky' wheat. Crop Science 43: 735–736.

Buteler M, Weaver DK & Peterson RKD (2009) Exploring the oviposition behavior of the wheat stem sawfly when encountering plants infested with cryptic conspecifics. Environmental Entomology 38: 1707–1715.

Buteler M, Weaver DK, Bruckner PL, Carson GR, Berg JE & Lamb P (2010) Using agronomic traits and semiochemical production in winter wheat cultivars to identify suitable trap crops for the wheat stem sawfly. Canadian Entomologist 142: 222–233.

Cook SM, Khan ZR & Pickett JA (2007) The use of push-pull strategies in integrated pest management. Annual Review of Entomology 52: 375–400.

Crofts HJ (1989) On defining a winter wheat. Euphytica 44: 225–234.

Degen T, Dillmann C, Marion-Poll F & Turlings TCJ (2004) High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. Plant Physiology 135: 1928–1938.

Dicke M (1999) Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods? Entomologia Experimentalis et Applicata 91: 131–142.

Dudareva N & Pichersky E (2008) Metabolic engineering of plant volatiles. Current Opinions in Biotechnology 19: 181–189.

Farstad C (1940) The Development of the Western Wheat Stem Sawfly (*Cephus cinctus* Nort) in Various Host Plants as an Index of Resistance. PhD Dissertation, Iowa State College, Ames, IA, USA.

Fu DL, Szúcs P, Yan LL, Helguera M, Skinner JS et al. (2005) Large deletions within the first intron in VRN-1 are associated with spring growth habit in barley and wheat. Molecular Genetics and Genomics 274: 442–443.

Grant MN (1980) Registration of Norstar winter wheat. Crop Science 20: 552.

Hattori M (1988) Host-plant factors responsible for oviposition behavior in the lima bean pod borer *Etiella zinckenella* Treitschke. Journal of Insect Physiology 34: 191–196.

Hites RA (1997) Gas chromatography mass spectrometry. Handbook of Instrumental Techniques for Analytical Chemistry (ed. by F Settle), pp. 609–626. Prentice Hall, Upper Saddle River, NJ, USA.

Hokkanen HMT (1991) Trap cropping in pest management. Annual Review of Entomology 36: 119–138.

Holmes ND & Peterson LK (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.

Khan ZR & Pickett JA (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 GM Gurr, SD Wratten & MA Altieri), pp. 155–164. Cornell University Press, Ithaca, NY, USA.

Knodel JJ, Beauzay PB, Eriksmoen ED & Pederson JD (2009) Pest management of wheat stem maggot (Diptera: Chloropidae) and wheat stem sawfly (Hymenoptera: Cephidae) using insecticides in spring wheat. Journal of Agricultural and Urban Entomology 26: 183–197.

Lou YG, Hua XY, Turlings TCJ, Cheng JA, Chen XX & Ye GY (2006) Differences in induced volatile emissions among rice varieties result in differential attraction and parasitism of *Nilaparvata lugens* eggs by the parasitoid *Anagrus nilaparvatae* in the field. Journal of Chemical Ecology 32: 2375–2387.

Miller RH, Elmasri S & Aljundi K (1993) Plant density and wheat stem sawfly (Hymenoptera: Cephidae) resistance in Syrian wheats. Bulletin of Entomological Research 83: 95–102.

Morrill WL & Kushnak GD (1996) Wheat stem sawfly (Hymenoptera: Cephidae) adaptation to winter wheat. Environmental Entomology 25: 1128–1132.

Morrill WL, Kushnak GD, Bruckner PL & Gabor JW (1994) Wheat stem sawfly damage rates of parasitism and overwintering survival in resistant wheat lines. Journal of Economic Entomology 87: 1373–1376.

Morrill WL, Weaver DK & Johnson GD (2001) Trap strip and field border modification for management of the wheat stem sawfly *Cephus cinctus* Nort (Hymenoptera: Cephidae). Journal of Entomological Science 36: 34–35.

Nansen C, Payton ME, Runyon JB, Weaver DK, Morrill WL & Sing SE (2005) Pre-harvest sampling plan for wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae) larvae in winter wheat fields. Canadian Entomologist 137: 602–614.

Nottingham SF (1988) Host-plant finding for oviposition by adult cabbage root fly *Delia radicum*. Journal of Insect Physiology 34: 227–234.

Pickett JA, Wadhams LJ & Woodcock CM (1997) Developing sustainable pest control from chemical ecology. Agricultural Ecosystems and Environment 64: 149–156.

Pickett JA, Bruce TJA, Chamberlain K, Hassanali A, Kahn ZR et al. (2006) Plant volatiles yielding new ways to exploit plant defence. Chemical Ecology: From Gene to Ecosystem (ed. by M Dicke & W Takken), pp. 161–173. Springer, Dordrecht, The Netherlands.

Piesik D, Weaver DK, Peck GE & Morrill WL (2006) Mechanically-injured wheat plants release greater amounts of linalool and linalool oxide. Journal of Plant Protection Research 46: 29–39.

Piesik D, Weaver DK, Runyon JB, Buteler M, Peck GE & Morrill WL (2008) Behavioural responses of wheat stem sawflies to wheat volatiles. Agricultural and Forest Entomology 10: 245–253.

Platt AW & Farstad C (1946) The reaction of wheat varieties to wheat stem sawfly attack. Scientific Agriculture 26: 231–247.

Ramaswamy SB (1988) Host finding by moths: sensory modalities and behaviors. Journal of Insect Physiology 34: 235–249.

SAS Institute (1998) The SAS System Version 7 for Windows. SAS Institute, Cary, NC, USA.

Seamans H (1928) The value of trap crops in the control of the wheat stem sawfly in Alberta. Annual Report of the Entomological Society of Ontario 5: 59–64.

Shanower TG & Hoelmer KA (2004) Biological control of the wheat stem sawflies: past and future. Journal of Agricultural and Urban Entomology 21: 197–221.

Shelton AM & Badenes-Perez FR (2006) Concepts and application of trap cropping in pest management. Annual Review of Entomology 51: 285–308.

Sherman JD, Weaver DK, Hofland ML, Buteler M, Lanning SP et al. (2010) Identification of novel QTL for sawfly resistance in wheat. Crop Science 50: 73–86.

Sunderman DW (1983) Registration of Neeley wheat. Crop Science 23: 187.

USDA NASS (United States Department of Agriculture, National Agricultural Statistics Service) (2010) Montana Agricultural Statistics, Vol. 47. USDA-NASS, Washington, DC, USA.

USDA-AMS, PVPO (United States Department of Agriculture-Agriculture Marketing Service, Plant Variety Protection Office) (1997) PVPO Certificate No. 9700399. ‘Morgan’. http://apps.ams.usda.gov/CMS//AdobeImages/009700399.pdf

Weaver DK, Sing SE, Runyon JB & Morrill WL (2004) Potential impact of cultural practices on wheat stem sawfly (Hymenoptera: Cephidae) and associated parasitoids. Journal of Agricultural and Urban Entomology 21: 271–287.

Weaver DK, Buteler M, Hofland ML, Runyon JB, Nansen C et al. (2009) Cultivar preferences of ovipositing wheat stem sawflies as influenced by the amount of volatile attractant. Journal of Economic Entomology 102: 1009–1017.

Weiss MJ & Morrill WL (1992) Wheat stem sawfly (Hymenoptera: Cephidae) revisited. American Entomologist 38: 241–245.

Witzgall P, Kirsch P & Cork A (2010) Sex pheromones and their impact on pest management. Journal of Chemical Ecology 36: 80–100.

Zadoks JC, Chang TT & Konsak CF (1974) A decimal code for growth stages of cereals. Weed Research 14: 15–21.