

Cultivar Preferences of Ovipositing Wheat Stem Sawflies as Influenced by the Amount of Volatile Attractant

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J. Econ. Entomol. 102(3): 1009–1017 (2009)

ABSTRACT The wheat stem sawfly, *Cephus cinctus* Norton, causes severe losses in wheat grown in the northern Great Plains. Much of the affected area is planted in monoculture with wheat, *Triticum aestivum* L., grown in large fields alternating yearly between crop and no-till fallow. The crop and fallow fields are adjacent. This cropping landscape creates pronounced edge effects of sawfly infestations and may be amenable to trap cropping using existing agricultural practices. The behavioral preference for two wheat varieties was assessed in the context of developing trap crops for this insect. In field nurseries, stem lodging assessments indicated that the cultivar ‘Conan’ was infrequently damaged, whereas ‘Reeder’ was often heavily damaged. In laboratory choice and no-choice tests, ‘Reeder’ was significantly preferred by ovipositing wheat stem sawfly females. These two cultivars did not differ significantly in height or developmental stage, factors known to impact sawfly preference. Although Conan received fewer eggs than Reeder in no-choice tests, oviposition was further reduced in choice tests, indicating that females clearly preferred Reeder. In field trials where the overall dimensions of the spatial structure in choice tests was varied, females always selected Reeder over Conan in alternating block, row, and interseeded planting scenarios. Reeder releases greater amounts of the attractive compound, (Z)-3-hexenyl acetate than Conan but is similar to Conan for three other known, behaviorally active volatile compounds. The results are discussed in terms of cultivar selection for large scale trap crop experiments for the wheat stem sawfly.

KEY WORDS trap crops, host preference, semiochemicals, (Z)-3-hexenyl acetate, attractant

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is a key pest of wheat, *Triticum aestivum* L., in the northern Great Plains of the United States and Canada. Annual losses caused by this insect are estimated at more than \$100 million per year in the affected region (Wahl et al. 2007), and with the current increase in wheat prices, yearly losses may be closer to \$200 million. Conventional management practices available for this insect provide limited control. The main tool available is the planting of solid-stemmed wheat resistant to lodging, but adoption is limited because of grower concerns about inconsistent performance. In addition, insecticides are not effective because all immature stages are within the plant and adult flight periods are often >1 mo. Recent trends in wheat cultivation on the northern Great Plains include increasing field size and greater adoption of no-till practices, which considerably alter as-

pects of wheat stem sawfly management (Runyon et al. 2002, Weaver et al. 2004). For example, planting larger fields and reducing tillage promotes a marked concentration of the wheat stem sawfly to the periphery of the crop (Nansen et al. 2005a, Weaver et al. 2005) and further enhances the “edge effect” that has long been reported as a characteristic of infestations (Criddle 1917). Edge effects have been a useful characteristic in development of sampling and management plans for this insect (Pesho et al. 1971, Nansen et al. 2005b). For example, perimeter trap crops were first suggested nearly a century ago (Criddle 1917, Farstad and Jacobson 1945) to intercept foraging adults that emerged from infested stubble in adjacent fallow fields (i.e., from the previous year’s crop). The trap may be planted with a resistant crop or removed from the field after the oviposition period to kill the developing larvae (Callenbach and Hansmeier 1945). Adoption of this practice was limited because of difficulty of implementation in small fields and inconsistent performance. Current cropping practices used in wheat monoculture suggest that the concept of trap crops should be reconsidered. For example, promising results were obtained in a study by Morrill et al. (2001), with a 24-m-wide trap strip of winter wheat protecting a larger area of spring wheat. This strategy is based on the premise that wheat stem sawfly adults

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oviposit preferentially in the taller winter wheat, which is planted earlier, rather than in the later maturing spring wheat field. This approach, coupled with other alternative control measures involving behavior modification, has great potential in wheat stem sawfly management. For example, cultivars of differing attractiveness could be used in a stimulo-deterrent diversionary strategy to manage wheat stem sawfly by using less attractive cultivars as crops, along with attractive ones as traps, therefore concentrating oviposition in the trap (Miller and Cowles 1990, Cook et al. 2007). Such "push-pull" strategies could be further enhanced by application of semiochemicals. For example, the use of aggregation or sex pheromones can increase movement of insect pests into the trap crop (Hokkanen 1991). Effectiveness of trap crops may also be increased by selecting plants that produce large quantities of attractant compounds (Agelopoulos et al. 1999, Khan et al. 2000). However, an understanding of the interactions between the specific organisms and their chemical ecology is fundamental to the success of such techniques (Pickett et al. 1997). Further knowledge of the mechanisms underlying host plant preference is essential for successful development of management practices that exploit insect behavior, such as trap cropping.

Host selection by insects can be viewed as a continuum between choosing their host from a distance using olfactory cues and host acceptance after contact (Bruce et al. 2005). Information available suggests that wheat stem sawfly oviposition behavior is most likely a catenary process involving different kinds of host finding cues and host acceptance cues (Ramaswamy 1988), similar to reported oviposition behaviors in other insects (Hattori 1988, Nottingham 1988). Within a host species, female wheat stem sawfly prefer the larger stems as oviposition sites (Holmes and Peterson 1960, Youtie and Johnson 1988, Morrill et al. 1992, Perez-Mendoza et al. 2006). Stem diameter seems to influence oviposition preference, but its importance is unclear given that stem diameter and height are highly correlated (Perez-Mendoza et al. 2006). Recent studies have suggested that release of volatile compounds from host plants provides cues for female wheat stem sawfly that may aid in the assessment of stems suitable for oviposition (Piesik et al. 2008). Several wheat volatiles that elicit behavioral activity from wheat stem sawfly have been identified: the green leaf volatiles, (Z)-3-hexenyl acetate and (Z)-3-hexenol, plus the monoterpenes, β -ocimene, and the carotenoid derivative, 6-methyl-5-hepten-2-one (Piesik et al. 2008).

The wheat stem sawfly will infest many members of the Poaceae, but they do exhibit oviposition preferences among species (Ainslie 1929). Moreover, Wall (1952) reported that wheat cultivars varied markedly in the percentage of damaged stems when exposed to wheat stem sawfly attack, although none were resistant to infestation. Observations from advanced yield trials field data show that certain wheat cultivars differ in the amount of lodging damage by wheat stem sawfly when planted in small plot nurseries (Lanning et al. 2007). These differences in damage, or lodging, could

be explained by differences in attractiveness to ovipositing females. However, although lodging caused by stem cutting by cryptic wheat stem sawfly larvae does provide a measure of relative host plant susceptibility, it is not a direct measure of the attractiveness of the cultivars. For example, larval mortality within the stems, primarily caused by parasitism, prevents stem lodging (Runyon et al. 2002, Buteler et al. 2008).

To examine factors influencing cultivar preference by female sawflies, we evaluated two spring wheat cultivars that differed in the amount of damage experienced in neighboring small plots in field nurseries. These two popular spring wheat cultivars grown in Montana are suitable hosts on which wheat stem sawfly can complete development; 'Reeder' typically has high levels of wheat stem sawfly damage, and 'Conan' has low levels of damage in nursery plots (Lanning et al. 2007). We first assessed the preference of ovipositing wheat stem sawfly for these cultivars in the field and in paired choice tests in the greenhouse. To investigate potential mechanisms underlying observed oviposition differences between cultivars, we compared their production of known behaviorally active volatiles and plant height, which is known influence wheat stem sawfly host choice. The characterization of variation in host preference for commonly grown varieties is fundamental in developing trap crops and allows for selection of cultivars most suitable for this use. We used these data to discuss the feasibility of this concept in dryland wheat cropping systems in Montana, because in practicality, trap cropping based on varieties commonly available and grown in the area will be more readily adopted than an approach based on exotic species or cultivars.

Materials and Methods

Greenhouse Preference Tests. *Source of Insects.* Adult wheat stem sawflies were obtained from field-collected, larval-cut wheat stems ("stubs"), each containing a larva in diapause. These were maintained in plastic zippered storage bags at 0–4°C for >100 d to facilitate completion of larval diapause. The stubs were subsequently placed in plastic Tupperware boxes (70 by 35 by 20 cm) held at room temperature (22–27°C) until adult emergence began 4–5 wk later. The boxes were opened daily, and emerging wheat stem sawflies were removed and placed in 2-liter Mason glass jars until they were used for experiments. Glass jars contained moistened filter paper and were supplemented with a sucrose solution. All bioassays were conducted with adults within 24 h after eclosion.

Plant Culture. Greenhouse experiments were performed at the Plant Growth Center, Montana State University. Spring wheat seeds of the cultivars Reeder or Conan were sown in tapered, square pots (13 by 13 by 13.5 cm) in a greenhouse with supplemental light (GE Multi-Vapor Lamps-model MVR1000/C/U, GE Lighting; General Electric, Cleveland, OH), as previously described in Piesik et al. (2008). The photoperiod was 15:9 (L:D) h. Daytime temperature was 22 $\pm$ 2°C, and the overnight temperature was 20 $\pm$ 2°C. The

relative humidity was ambient, typically ranging from 20 to 40%. Soil used consisted of equal parts of Montana State University Plant Growth Center 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). The soil pH was 6.15 and the electrical conductivity was 0.85 dS/m. Wheat plants were watered three to four times weekly and fertilized with Peters General Purpose Fertilizer (J. R. Peters, Allentown, PA) at 100 ppm in aqueous solution twice each week. Fertilizing started when plants reached a developmental stage of Zadoks 13 (three unfolded leaves) (Zadoks et al. 1974).

Plants were used at the developmental stage of Zadoks 32 (two nodes visible) and Zadoks 49 (when the awns of the developing head are first visible). These two stages were chosen for because they represent the youngest and oldest host plant stages susceptible to wheat stem sawfly under field conditions.

Choice and No-Choice Tests. To investigate wheat stem sawfly discrimination between cultivars, Conan and Reeder were simultaneously presented in greenhouse choice tests. In addition, a no-choice test was conducted with each cultivar. Both tests were conducted in screened cages (91.4 by 66.7 by 91.4 cm) with 530- μ m mesh openings (BioQuip Products, Rancho Dominguez, CA). Each cage contained paired, evenly spaced square tapered pots (13 by 13 by 13.5 cm), each one containing three plants. Ten females and five males were released in each cage to allow mating and oviposition for 2 d. Immediately afterward, the pots were removed from the cages, and stems were dissected to count eggs.

Eleven individual replicates of the choice tests were conducted with plants in an early developmental stage (Zadoks 32) and 11 with plants at a later developmental stage (Zadoks 49). Four no-choice replicates with plants at the early and three with plants at the later developmental stage were also conducted for each cultivar. Plants usually had between two and four stems, depending on the developmental stage, because of sequential tillering in the host plant.

Field Experiments. Three experiments were set up in the field to compare oviposition preference for the two spring wheat cultivars at different spatial scales (Fig. 1). The first experiment consisted of a block trial with six replicates of alternating Conan and Reeder blocks that were 6.71 m long and 3.66 m wide (Fig. 1a). The second experiment consisted of a row trial with 10 blocks, 6.71 m long and 3.66 m wide, where single rows of each cultivar were alternated (Fig. 1b). The third experiment consisted of eight 124 by 60-m blocks, where the two cultivars were interseeded at equal densities.

Row and Block Trials With Alternating Cultivars. The row and block trials were conducted on a private farm, near Loma, Chouteau County, MT, in 2003 (latitude 48°05.782' N, longitude 110°27.174' W) and in 2004 (latitude 48°05.814' N, longitude 110°27.491' W). The experiments were planted within fields of winter wheat with a history of wheat stem sawfly infestation, and the 2004 experiments were conducted 0.39 km

northwest of the site used in 2003. The soil type was clay, with a pH of 6, an average organic matter content of 1.2%, Olsen available phosphorus of 27 mg/kg, and exchangeable potassium of 272 mg/kg in the surface 15 cm in 2003. In 2004, pH was 5.4, organic matter content was 1.4%, available phosphorus was 35 mg/kg, and exchangeable potassium was 409 mg/kg. Spring wheat was seeded on 26 April 2003 and on the same date in 2004 at sites previously cropped to wheat under no-till management at a rate of 67 kg/ha. Plots were harvested on 11 August 2003 and 9 August 2004. Granular fertilizer was applied at seeding, at a rate of 77 kg/ha nitrogen, 44 kg/ha phosphorus (P_2O_5), and 27.5 kg/ha potassium (K_2O). Plant samples were collected three times during the growing season: 13 June, 30 June, and 14 July 2003 and 16 June, 23 June, and 8 July 2004. The first sampling event occurred during stem elongation (approximately Zadoks 31–32) and the last one during flowering (approximately Zadoks 65). On the sampling dates in June, early in the oviposition period, the stems contained mainly eggs. Stems collected subsequently in July contained predominantly larvae. Samples were collected on different dates to determine the relative attractiveness of each cultivar at different stages spanning the wheat stem sawfly oviposition flight period.

For the block trials, three random samples were collected at each block for each variety and on each sampling date ($n = 18$). For the row trials, two random samples were collected at each row for each variety and on each sampling date ($n = 20$). Samples consisted of all spring wheat plants in a 30-cm length of row.

Block Trials With Interseeded Wheat Cultivars. The third experiment was conducted on a private farm north of Havre, Hill County, MT, in 2008 (latitude 48°49.818' N, longitude 110°06.688' W). The experiment was planted within a large field of winter wheat with a history of wheat stem sawfly infestation and was encircled by a smaller border of spring wheat. The 2008 experiment was conducted 85 km northeast of the sites used in 2003 and 2004. The soil type was clay loam, with a pH of 6.4, an average organic matter content of 0.9%, Olsen available phosphorus of 18 mg/kg, and exchangeable potassium of 272 mg/kg at 15 cm from the surface for 2008. A 50:50 interseeding of Reeder and Conan was planted on 14 May, at a site previously cropped to wheat under no-till management. The overall seeding rate was 270 seed/m², based on Conan seed at 70 kg/ha and Reeder seed at 52 kg/ha. Granular fertilizer was applied before seeding on 18 April, at a rate of 56 kg/ha nitrogen, 22.4 kg/ha phosphorus (P_2O_5), and 11.2 kg/ha potassium (K_2O). Plant samples were collected twice during the growing season: 1 July (Zadoks 41) and 22 July (Zadoks 60). Two random samples within each block were collected on each sampling date. Samples consisted of all spring wheat plants in 15 cm of row. One cultivar has a glaucous stem surface and the other does not. This trait was used to identify the plants from each cultivar in each sample. The degree of glaucousness does not correlate with the level of wheat stem sawfly infestation (D.K.W. et al., unpublished data).

Wheat stem sawfly infestation was determined by

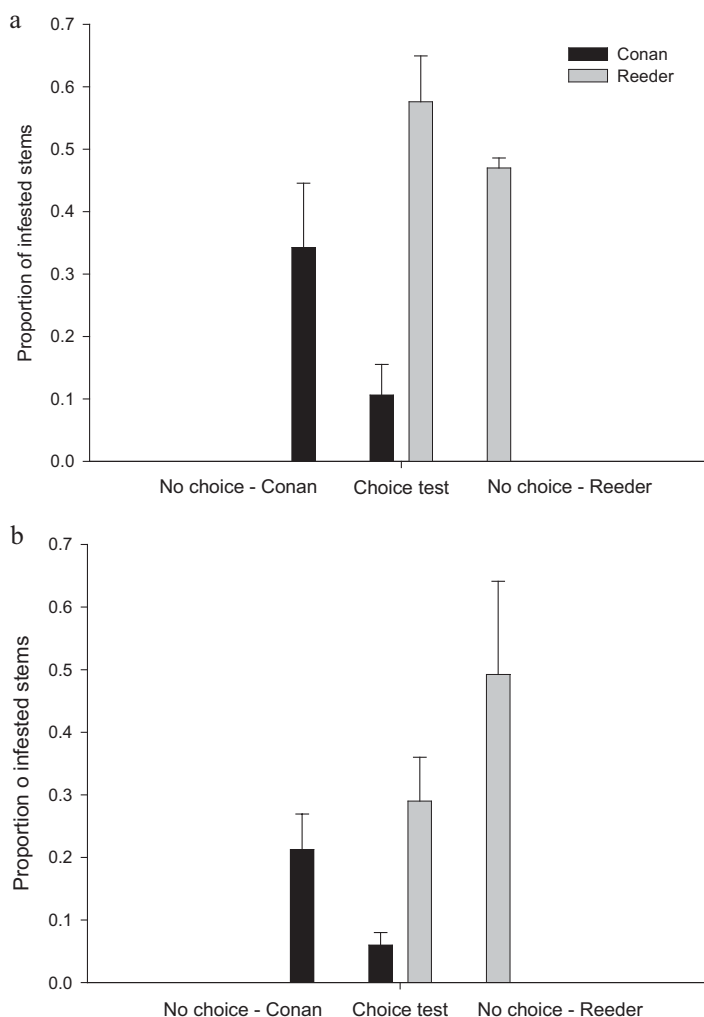


Fig. 1. Infestation in Conan and Reeder, expressed as proportion of infested stems in a cage, in choice and no-choice tests. Two experiments were conducted, with plants at growth stage (a) Zadoks 32 and (b) Zadoks 49.

the presence of eggs, larvae, or frass resulting from the feeding of larvae within the stem, according to Runyon et al. (2002). The number of immatures and percentage of infested stems per sample were recorded and compared between cultivars.

Volatile Collection and Analysis. To quantify volatile compounds, intact wheat plants of Conan and Reeder cultivars were placed in collection chambers in a volatile collection system as previously described in Piesik et al. (2006). Briefly, wheat volatiles were collected by pulling air through traps for 8 h between 1000 and 1800 hours (under an ambient, supplemented photophase). The main stem on each plant was enclosed in a glass volatile collection chamber (40 mm diameter by 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 OD by 76 mm long; Analytical Research Systems, Gainesville, FL) containing 30 mg of Super-Q adsorbent (Alltech Associates, Deerfield, IL) were inserted into each vol-

atile collector port. Purified, humidified air was delivered at a rate of 1.0 liter/min over the stem, and the flow and pressure were maintained by a regulated vacuum pump. A Teflon sleeve encircled the base of the stem and was taped to the glass volatile collection system tube to prevent outside air from entering the system.

The trap filters were eluted with 200 μ l 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, *trans*-2-nonene (Sigma-Aldrich, Milwaukee, WI), in a hexane solution was added. Samples were analyzed by gas chromatography-mass spectrometry (GC-MS) under the following conditions: fused silica-column (30 m by 0.25 mm) with a 0.25- μ m DB-5 stationary phase, held at 50°C for 4 min after injection into a 250°C port and increased at 5°C/min to 160°C, followed by a 25°C/min ramp to 280°C for 3 min, with He carrier gas maintained at a flow rate of 1.2 ml/min. The samples were injected onto the column in pulsed-

Table 1. Plant height (mean $\pm$ SE), number of tillers (mean $\pm$ SE), and number of wheat stem sawfly eggs per plant (mean $\pm$ SE) in choice tests using two spring wheat cultivars at two developmental stages

Cultivar ^a	Height (cm)		No. tillers		No. eggs per plant	
	Zadoks 32 ^a	Zadoks 49 ^a	Zadoks 32 ^a	Zadoks 49 ^a	Zadoks 32 ^a	Zadoks 49 ^a
Conan	15.90 $\pm$ 0.76a	29.29 $\pm$ 0.82a	2.03 $\pm$ 0.14a	2.26 $\pm$ 0.16a	0.22 $\pm$ 0.09a	0.30 $\pm$ 0.11a
Reeder	17.16 $\pm$ 0.72a	30.38 $\pm$ 0.79a	2.19 $\pm$ 0.16a	2.70 $\pm$ 0.16a	2.56 $\pm$ 0.50b	2.61 $\pm$ 0.52b

Comparisons are between rows of same column. Rows with different letters within a column indicate significant differences ($P < 0.05$).

^a $n = 33$ for each growth stage of both varieties.

splitless mode, with an initial pressure of 0.84 kg/cm for 1 min. A temperature of 300°C was maintained on the transfer line to the MSD. Eluted compounds were detected by an Agilent 5973 mass selective detector (150°C) in electron-impact ionization mode scanning masses 10–300. Samples were analyzed for (Z)-3-hexenyl acetate, (Z)-3-hexenol, (E)- β -ocimene, and 6-methyl-5-hepten-2-one content. The identities of volatile compounds were determined from the comparison to the mass spectra and retention times of authentic standards. Commercial samples of the volatile compounds were obtained from Sigma-Aldrich (Milwaukee, WI) [(Z)-3-hexenol, 6-methyl-5-hepten-2-one] and Bedoukian Research (Danbury, CT) [(Z)-3-hexenyl acetate]. (E)- β -ocimene was synthesized in the laboratory at the Rothamsted Research Center, Hertfordshire, United Kingdom. Quantification of compounds was made relative to the internal standard.

All peaks considered were examined for purity while verifying their identity, and peaks that were not reliably quantified were not included in the analysis, which happened occasionally for (Z)-3-hexen-1-ol. Each experiment consisted of three plants of each cultivar randomly distributed within the volatile collection system, whereas a control consisted of the airspace in an open volatile collection chamber above a pot containing soil only. The experiment was replicated three times with plants at Zadoks 32 and three times with plants at Zadoks 49.

Data Analyses. Differences between the number of eggs per plant for each cultivar in the greenhouse tests were analyzed using generalized linear mixed models with SAS PROC MIXED (SAS Institute 1998). Replicate was included as a random factor, and plant height was included as a covariate in the model. The variables eggs per plant and eggs per cage were transformed using the $\log_{10}(x + 0.5)$ before analysis to achieve normality of the residuals. The proportion of infested stems per cage in each cultivar was analyzed using a generalized linear mixed model in the SAS macro GLIMMIX (Littell et al. 2006). Replicate was included as a random factor. Data are presented as the mean $\pm$ SEM of the untransformed data. Mean plant heights and the mean number of tillers between cultivars were separated with t -tests (PROC TTEST; SAS Institute 1998).

Two variables were analyzed to study infestation data from the field trials. To compare the number of samples containing wheat stem sawfly larvae in the different cultivars, infestation was modeled as a binary response variable (1 = infested sample, 0 = not infested sample). Data were logit transformed and an-

alyzed with the GLIMMIX macro of SAS in the general mixed model analysis with binary error structure (Littell et al. 2006). Differences in percent infestation per sample were analyzed using generalized linear models in SAS PROC MIXED (SAS Institute 1998). Each type of experimental plot (rows or blocks), representing the spatial scale of the experiment, was analyzed separately. Cultivar, year, and sampling date were included in the model as fixed effects. Sampling date was also included as the repeated measures factor. The compound symmetric covariance structure was fitted to the model. Replicate was not included in the model because it was not significant in contributing to explain variability in infestation. Percent infestation was transformed using the $\log_{10}(x + 0.5)$ before analysis to stabilize homogeneity of variance. The 2008 experiment was analyzed separately with cultivar, date, and replicate (block) as fixed effects. Sampling date was also included as the repeated measures factor in this model.

The volatile collection data were subjected to a factorial multivariate analysis of variance (MANOVA; PROC GLM; SAS Institute 1998) to determine differences among amounts of behaviorally active compounds emitted by the cultivars at the two developmental stages. The Wilks' lambda test statistic was used to discriminate significant main effects in the MANOVA. Univariate ANOVAs were conducted to further study potential differences between the cultivars for each compound. The data were square-root transformed to better meet the assumption of normality for the distribution. Amounts are presented as nanograms per plant per hour and amounts corrected for plant biomass. Data are presented as mean $\pm$ SEM of the untransformed data.

Results

Greenhouse Preference Tests. In choice tests, cultivar was a significant factor explaining variability in the number of eggs per plant at both growth stages (Zadoks 32: $F = 40.26$, $df = 1,53$, $P < 0.0001$; Zadoks 49: $F = 52.96$, $df = 1,53$, $P < 0.0001$). The number of eggs per plant was significantly greater in Reeder than in Conan (Table 1). Plant height did not contribute to variation in the number of eggs per plant when both cultivars were present in choice tests (Zadoks 32: $F = 1.51$, $df = 1,53$, $P = 0.22$; Zadoks 49: $F = 2.10$, $df = 1,53$, $P = 0.15$). Moreover, Conan and Reeder plants did not differ significantly in plant height at Zadoks 32 ($t = -1.19$, $df = 62$, $P = 0.24$) or Zadoks 49 ($t = -1.04$, $df = 57.2$, $P = 0.30$; Table 1). Conan and Reeder did not

Table 2. Proportion of infested stems (mean $\pm$ SE) and number of eggs per cage (mean $\pm$ SE) in choice tests using two spring wheat cultivars at two developmental stages

Cultivar ^a	Proportion of infested stems		No. eggs per cage	
	Zadoks 32 ^a	Zadoks 49 ^a	Zadoks 32 ^a	Zadoks 49 ^a
Conan	0.11 $\pm$ 0.05a	0.06 $\pm$ 0.02a	0.64 $\pm$ 0.31a	1.0 $\pm$ 0.5a
Reeder	0.58 $\pm$ 0.07b	0.29 $\pm$ 0.07b	7.36 $\pm$ 1.67b	8.09 $\pm$ 2.18b

Comparisons are between rows of same column. Rows with different letters within a column indicate significant differences ($P < 0.05$).

^a $n = 33$ for each growth stage of both varieties.

differ in the number of tillers per plant (Zadoks 32: $t = -0.73$, $df = 62$, $P = 0.47$; Zadoks 49: $t = -1.92$, $df = 62$, $P = 0.06$). The number of eggs per cage was significantly greater in Reeder than in Conan pots (Zadoks 32: $F = 69.44$, $df = 1,10$, $P < 0.0001$; Zadoks 49: $F = 25.38$, $df = 1,10$, $P = 0.0005$; Table 2). The

proportion of infested stems per cage in choice tests (Table 2; Zadoks 32: $F = 20.74$, $df = 1,10$, $P = 0.001$; Zadoks 49: $F = 10.4$, $df = 1,10$, $P = 0.009$) and in no-choice tests (Fig. 2) was significantly lower in Conan plants than in Reeder plants at both stages tested. However, the proportion of infested stems in Conan plants in no-choice tests was greater than in choice tests (Fig. 2).

Field Experiments. Block and Row Trials With Alternating Wheat Cultivars. Wheat stem sawfly infestation percentages for Conan and Reeder differed significantly in the row ($F = 33.72$, $df = 1,37$, $P < 0.0001$) and block field trials ($F = 48.75$, $df = 1,21$, $P < 0.0001$). Overall infestation percentages were greater in Reeder than in Conan samples (Fig. 3). There was also a significant difference between the two cultivars in the number of samples in which infestation was observed over time (row trials: $F = 30.45$, $df = 1,50.42$, $P < 0.0001$; block trials: $F = 37.70$, $df = 1,24.27$, $P <$

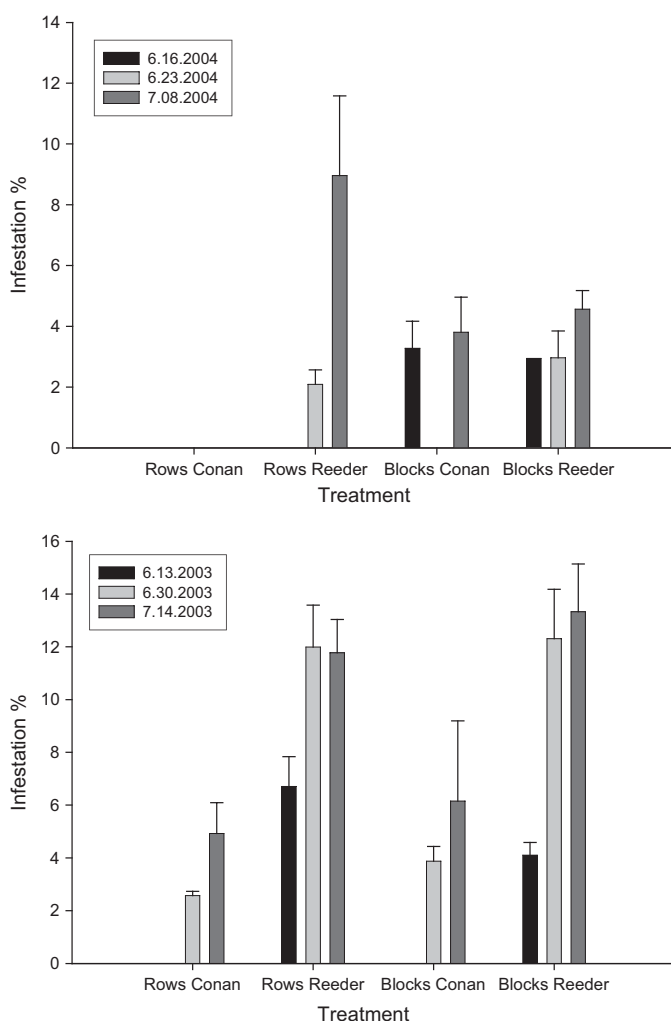


Fig. 2. Percent infestation in plant samples that wheat stem sawfly was observed. Data from the field experiments conducted in 2003 and 2004 are presented.

Table 3. Number of plant samples that were infested by wheat stem sawfly in collections through the growing season in field experiments in 2003 and in 2004

Experiment	Cultivar ^a	2003			2004		
		13 June ^a	30 June ^a	14 July ^a	16 June ^a	23 July ^a	8 July ^a
Rows	Conan	0	5	8	0	0	5
	Reeder	13	18	20	0	2	13
Blocks	Conan	0	4	4	2	0	8
	Reeder	16	18	17	1	2	11

^a $n = 20$ samples from the row plots and 18 samples from the block plots for each cultivar on each sampling date.

0.0001; Table 3). There was a significant effect of sampling date on percent infestation (row trials: $F = 22.56$, $df = 2,78$, $P < 0.0001$; block trials: $F = 23.12$, $df = 2,34$, $P < 0.0001$), as well as on the number of infested samples (row trials: $F = 18.41$, $df = 2,234$, $P < 0.0001$; block trials: $F = 17.46$, $df = 2,211$, $P < 0.0001$). Overall, infestation increased over time, as the adult flight progressed, and infestation was greater in Reeder whether the cultivars were planted in adjacent rows or in blocks (Table 3). There was also a significant effect of year (2003 versus 2004) in infestation percentages (row trials: $F = 24.69$, $df = 1,37$, $P < 0.0001$; block trials: $F = 25.75$, $df = 1,21$, $P < 0.0001$), as well as in the number of infested samples (row trials: $F = 25.71$, $df = 1,46.13$, $P < 0.0001$; block trials: $F = 28.53$, $df = 1,84.65$, $P < 0.0001$). In 2003, most or all of the Reeder samples were infested, whereas only a few of the Conan samples contained wheat stem sawflies (Table 3). In 2004, the number of infested samples in the field was very low compared to 2003. Nevertheless, there were always a greater number of Reeder samples that were infested compared with Conan, except for the first sampling date in 2004 (Table 3).

Block Trials With Interseeded Wheat Cultivars. Wheat stem sawfly infestation in 2008 in North Havre was greater than for the earlier experiments in Loma, and all of the plant samples collected were infested. There was a significant difference in infestation between Conan and Reeder plants in the field experiment in 2008 ($F = 139.49$, $df = 1,7$, $P < 0.0001$) and a difference in infestation across sampling dates ($F = 10.36$, $df = 1,15$, $P = 0.006$) and no block (rep) effect ($F = 1.35$, $df = 7,7$, $P = 0.35$). The infestation percentage per sample was significantly greater in Reeder

Table 4. Percent wheat stem sawfly infestation in Conan and Reeder stems from samples taken in large interseeded blocks during the 2008 field experiment

Cultivar	Infestation %	
	1 July ^a	22 July ^a
Conan	9.2 ± 3.0a	8.6 ± 2.6a
Reeder	41.7 ± 5.2b	65.0 ± 3.3b

Comparisons are between rows of same column. Rows with different letters within a column indicate significant differences ($P < 0.05$).

^a $n = 16$ samples for each cultivar on each collection date.

Table 5. Amount (mean ± SE) of three behaviorally active volatile compounds emitted by Conan and Reeder spring wheat at two developmental stages (ng g⁻¹ aboveground biomass h⁻¹)

Cultivar	Zadoks stage ^a	Compound		
		(Z)-3-hexenyl acetate	(E)- β -ocimene	6-methyl-5-hepten-2-one
Conan	32	0.32 ± 0.07a	0.67 ± 0.20a	0.08 ± 0.01a
	49	0.03 ± 0.01a	0.09 ± 0.04a	0.04 ± 0.01a
Reeder	32	0.92 ± 0.31b	0.25 ± 0.04a	0.07 ± 0.01a
	49	0.38 ± 0.11b	0.16 ± 0.05a	0.05 ± 0.01a

Comparisons are made within column and stage. Rows with different letters within column and stage are significantly different ($P < 0.05$).

^a $n = 18$ for each cultivar at each Zadoks stage.

than in Conan stems across blocks and sampling dates (Table 4).

Emission of Behaviorally Active Volatiles. There was an overall significant difference in the amount of volatile compounds produced by the two cultivars, as indicated by multivariate ANOVA (amount corrected for biomass [Table 5]: Wilks' Lambda = 0.76, $df = 3,53$, $F = 5.45$, $P = 0.002$; amount per plant [Table 6]: Wilks' Lambda = 0.73, $df = 5,53$, $F = 6.60$, $P = 0.0009$). There was also a significant difference between developmental stages in the amounts produced (amount corrected for biomass: Wilks' Lambda = 0.63, $df = 3,53$, $F = 10.5$, $P < 0.0001$; amount per plant: Wilks' Lambda = 0.57, $df = 3,53$, $F = 13.38$, $P < 0.0001$), but a significant interaction between stage and date of collection prevented further analysis of this main factor (amount corrected for biomass: Wilks' Lambda = 0.73, $df = 3,53$, $F = 6.56$, $P = 0.0009$; amount per plant: Wilks' Lambda = 0.76, $df = 3,53$, $F = 6.56$, $P = 0.0007$).

There was a significant difference in the amount of (Z)-3-hexenyl acetate released by different cultivars, as indicated by univariate ANOVA (amount corrected for biomass: $F = 8.62$, $df = 1$, $P = 0.0048$; amount per plant: $F = 11.18$, $df = 1$, $P = 0.0015$) and no significant differences in the amounts of 6-methyl-5-hepten-2-one (amount corrected for biomass: $F = 1.5$, $df = 1$, $P = 0.23$; amount per plant: $F = 2.1$, $df = 1$, $P = 0.15$) or β -ocimene emitted (amount corrected for biomass: $F = 3.80$, $df = 1$, $P = 0.06$; amount per plant: $F = 3.28$,

Table 6. Amount (mean ± SE) of three behaviorally active volatile compounds emitted by Conan and Reeder spring wheat at two developmental stages (ng plant⁻¹ h⁻¹)

Cultivar	Zadoks stage ^a	Compound		
		(Z)-3-hexenyl acetate	(E)- β -ocimene	6-methyl-5-hepten-2-one
Conan	32	1.43 ± 0.30a	2.74 ± 0.70a	0.35 ± 0.04a
	49	0.15 ± 0.03a	0.55 ± 0.29a	0.21 ± 0.03a
Reeder	32	4.26 ± 1.41b	1.2 ± 0.19a	0.31 ± 0.05a
	49	1.94 ± 0.66b	0.84 ± 0.30a	0.25 ± 0.04a

Comparisons are made within column and stage. Rows with different letters within column and stage are significantly different ($P < 0.05$).

^a $n = 18$ for each cultivar at each Zadoks stage.

df = 1, $P = 0.08$) 'Reeder' plants produced significantly greater amounts of (Z)-3-hexenyl acetate than Conan at both stages tested (Tables 5 and 6 for amounts corrected and uncorrected for biomass, respectively).

Discussion

In both greenhouse and field choice tests, wheat stem sawflies overwhelmingly preferred the wheat cultivar Reeder over Conan. This clear choice for Reeder persisted at different spatial scales whether cultivars were grown in randomized blocks, alternating rows, or intermixed in the same field (Figs. 1 and 2; Tables 3 and 4). Greater infestation was observed in Conan plants in no-choice tests compared with choice tests, confirming that Conan is suitable for infestation and is used when there is no other suitable host available.

These data showed that foraging wheat stem sawflies are able to distinguish between Reeder and Conan. Like other insect herbivores, it is likely that wheat stem sawflies exploit some combination of olfactory, visual, and contact cues when selecting host plants (Bruce et al. 2005). Plant height and developmental stage are known to influence host plant selection by wheat stem sawfly (Holmes and Peterson 1960), but we compared cultivars of the same age for which there were no significant differences in height or number of tillers (Table 1) that could potentially confound varietal differences in preference. Evidence suggested that wheat stem sawflies assess the suitability of potential hosts by walking up and down the length of the host stem while tapping it with their antennae and abdomen (Criddle 1917). However, it is known that wheat stem sawflies are attracted to several volatile compounds released by wheat (Piesik et al. 2008). Moreover, we found that Reeder and Conan plants differed in the emission of one of these behaviorally active volatiles; Reeder plants emit greater amounts of the attractive (Z)-3-hexenyl acetate at both stages tested, thus providing a plausible mechanism by which wheat stem sawfly distinguish between these cultivars. These results further support the hypothesis that semiochemicals play an important role in oviposition by wheat stem sawfly.

Collectively, these findings suggest that management of this pest may be improved through the exploitation or manipulation of host plant semiochemicals. For example, trap cropping could be optimized through the careful selection of cultivars differing in attractiveness to ovipositing females. Based on this study, the less attractive Conan could be planted as the primary crop adjacent to the more attractive Reeder as the trap crop. It is possible that by screening additional wheat cultivars for wheat stem sawfly attractiveness, either through behavioral or volatile analysis, the selection of crop/trap crop combinations could be further refined to optimize crop protection. Additionally, this system could also be enhanced using synthetic kairomones or wheat stem sawfly pheromones (Bartelt et al. 2002, Cossé et al. 2002) applied to the trap.

Previous findings showed that wheat stem sawflies preferentially infested winter wheat in a perimeter

trap protecting spring wheat, which indicates that trap cropping has the potential to manage wheat stem sawflies (Morrill et al. 2001). However, growers have been slow to adopt this approach, perhaps because of the inconsistency of results when the selection of spring wheat cultivars that were destined for large areas was based solely on agronomic traits, without consideration of relative preferences of wheat stem sawflies. Results from this study suggest that trap crops could further be enhanced by the use of cultivars differing in attractiveness to ovipositing wheat stem sawfly females and the exploitation of semiochemicals involved in host selection. This study provides the basis for further large-scale efforts on differing preference among wheat cultivars, which could lead to management practices involving the stimulo-deterrent diversionary strategy. These results will allow consideration of the potential of semiochemical augmentation in wheat crops. Additional study on behavioral manipulation is especially encouraged given that very large fields are currently planted next to infested stubble from fallow fields, defining a clear boundary that all foraging adults must pass through. To achieve this, it is tantamount to have a variety like Conan that is less preferred as a baseline to develop various forms of attraction for perimeter deployment. However, it is critical to note that large-scale monocultures of both Conan and Reeder are comparably infested in areas of significant pest pressure. Thus, it is a phenomenon of relative preference that is being exploited and not absolute antixenosis. In addition, the development of recombinant inbred lines using these two cultivars as parents has shown that oviposition preference is heritable and genetic markers can be used to assess this potential preference (D.K.W. et al., unpublished data). This will allow for the ready development of new cultivars using novel lines that are either attractive or unattractive, greatly expanding capability in developing trap crops. If the practice of surrounding unattractive crops with small areas of attractive cultivars is adopted on a landscape scale over many hectares, this may greatly reduce damage in wheat in the northern Great Plains.

Acknowledgments

This research was supported using funds from USDA-Western SARE award SW07-025, several USDA Special Research Grants entitled "Novel semiochemical- and pathogen-based management strategies for wheat stem sawfly," by the Montana Wheat and Barley Committee, and by the Montana Board of Research and Commercialization Technology. The authors thank K. Chamberlain for providing (*E*)- β -ocimene.

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Received 11 November 2008; accepted 2 March 2009.