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ORIGINAL ARTICLE

Host plant quantitative trait loci affect specific behavioral sequences in oviposition by a stem-mining insect

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Abstract

Key message **Genetic diversity in quantitative loci associated with plant traits used by insects as cues for host selection can influence oviposition behavior and maternal choice.**

Abstract Host plant selection for oviposition is an important determinant of progeny performance and survival for phytophagous insects. Specific cues from the plant influence insect oviposition behavior; but, to date, no set of host plant quantitative trait loci (QTLs) have been shown to have an effect on behavioral sequences leading to oviposition. Three QTLs in wheat (*Triticum aestivum* L.) have been identified as influencing resistance to the wheat stem sawfly (WSS) (*Cephus cinctus* Norton). Wheat near-isogenic lines (NILs) for each of the three QTLs were used to test whether foraging WSS were able to discriminate variation in plant cues resulting from allelic changes. A QTL on chromosome 3B (*Qss-msub-3BL)* previously associated with stem solidness and larval antibiosis was shown to affect WSS oviposition behavior, host preference, and field infestation. Decreased preference for oviposition was also related to a QTL allele on chromosome 2D (*Qwss.msub-2D*). A QTL on chromosome 4A (*Qwss.msub-4A.1*) affected host plant attractiveness to foraging females, but did not change oviposition preference after females landed on the stem. These findings show that oviposition decisions regarding potential plant hosts require WSS females to discriminate signals from the plant associated with allelic variation at host plant quantitative loci. Allele types in a host plant QTL associated with differential survival of immature progeny can affect maternal choices for oviposition. The multidisciplinary approach used here may lead to the identification of plant genes with important community consequences, and may complement the use of antibiosis due to solid stems to control the wheat stem sawfly in agroecosystems.

Abbreviations

NIL	Near-isogenic lines
QTL	Quantitative trait loci
RIL	Recombinant inbred lines
WSS	Wheat stem sawfly

Introduction

Plant traits that influence host selection for oviposition in phytophagous insects vary among and within plant species due to genetic and environmental factors. Female insects react to variability among host plants by showing a host preference hierarchy that often reflects adaptation for optimal host selection and utilization (Gripenberg et al. 2010; Mayhew 1997; Thompson 1983). Genetic variation in host species controlling insect oviposition influences ecology (e.g., host range, population dynamics) and evolution of the host and the insect. It may also allow for new opportunities to use behavioral manipulation methods to improve insect management in agroecosystems.

Several studies have identified host plant quantitative trait loci (QTLs) associated with insect oviposition

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preference (Alam and Cohen 1998; Lucatti et al. 2014; Satish et al. 2009; Sherman et al. 2010; Tao et al. 2003) and immature performance and survival (Rector et al. 2000; Sagredo et al. 2009; Tao et al. 2003; Varella et al. 2015; Yang et al. 2014). Oviposition preference may include long-distance detection of suitable plants, with subsequent physical and sensory evaluation of the host after the insect has alighted on the plant. Ideally, females should select plants for oviposition which will optimize larval performance and survival. However, no study has linked host plant QTLs to behavioral sequences leading to insect oviposition or that maternal oviposition choices for specific allele types at a plant locus are associated with differential survival of immature offspring.

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae) (WSS) was originally identified as a pest of many large-stemmed grass species in western US (Ainslie 1920). As European settlers began to farm the shortgrass prairie, large areas of native grasses were displaced to cultivate wheat, *Triticum aestivum* L. Shortly after wheat crops were planted, *C. cinctus* populations adapted to this new and widely distributed plant host (Fletcher 1896). This resulted in the common name for the insect to change from 'western grass stem sawfly' to 'wheat stem sawfly' (Ainslie 1929). The WSS is now a major pest of wheat in the northern Great Plains of North America (Beres et al. 2011). Adults emerge from wheat stubs from May to July and copulation occurs soon after emergence (Criddle 1922). Females lay eggs inside wheat stems and the larvae feed on parenchymal tissues and bore through vascular bundles of the plant (Macedo et al. 2005; Morrill et al. 1992, 1994). When the plant is nearly mature, the larva cuts the base of the stem in preparation for overwintering diapause (Ainslie 1929). Infested stems show reduced grain quality and yield; cut stems usually lodge and are not harvested (Beres et al. 2007; Morrill et al. 1992, 1994).

Although mobile within a wheat stem, a WSS larva is not capable of switching hosts; so, maternal choices during host plant selection are important determinants of progeny fitness and survival. Thus, female choices for oviposition are expected to be under strong selective forces to optimize host selection and utilization (Jaenike 1978). Not surprisingly, host selection by WSS is a multi-step process orchestrated by several plant cues (Buteler et al. 2009). Pre-alighting behaviors are controlled by visual and long-range chemical cues (Piesik et al. 2008; Weaver et al. 2009), while post-alighting behavioral displays are mainly driven by contact cues (Buteler et al. 2009). Utilization of plant cues for host selection by gravid females suggests that antixenosis mechanisms can influence WSS–host plant interaction. Alteration of plant traits used as cues could reduce infestation and, consequently, decrease insect damage in agroecosystems.

The selection of stems that are suitable for oviposition by female WSS is likely to be influenced by genes in the host plant. The ability to genetically differentiate the factors controlling this interaction exists in wheat due to the availability of molecular tools that do not exist for the native grasses. In this regard, Weaver et al. (2009) reported that spring wheat cultivars grown in Montana vary in attractiveness to ovipositing WSS females. A cross between a cultivar attractive to females, 'Reeder' (PI 613586), and an unattractive cultivar, 'Conan' (WestBred, LLC), revealed three QTLs controlling the number of eggs deposited in the stem (Sherman et al. 2010). These QTLs are designated *Qwss.msub-2D*, *Qwss.msub-4A.1*, and *Qwss.msub-4A.2*. The Reeder alleles at two QTLs were associated with greater oviposition, while *Qwss.msub-4A.2* was not.

Researchers have discovered significant diversity within wheat cultivars for susceptibility to damage caused by WSS larvae. An important early discovery was that while all wheat cultivars grown in the Great Plains had hollow stems, some wheat landrace accessions had pith-filled or solid stems (Platt et al. 1948). Larvae of WSS are unable to thrive in solid-stem wheat (Holmes and Peterson 1962), resulting in less damage and decreased overwintering success, as well as reduced fecundity in subsequent years (Cárcamo et al. 2005). Thus, the solid-stem trait was quickly adopted as the primary control measure for WSS. This trait has been found to be under simple genetic control with a single QTL on chromosome 3B, *Qss.msub-3BL*, controlling most of the variation (Cook et al. 2004; Kalous et al. 2011; Houshmand et al. 2007; Sherman et al. 2010; Varella et al. 2015). The solid-stem allele commonly used for resistance is derived from the Portuguese landrace S-615 (Platt et al. 1948). Subsequently, research showed that a different allele at *Qss.msub.3BL* from the cultivar Conan not only affected stem solidness and decreased larval survival, but also reduced the number of stems selected for oviposition (Talbert et al. 2014).

The existence of molecular markers for the QTLs affecting oviposition preference in wheat allowed the development of near-isogenic lines (NILs) for *Qss.msub-3BL*, *Qwss.msub-2D*, and *Qwss.msub-4A.1*. These NILs were used to assess the role of specific wheat QTLs in the oviposition behavior of WSS. Interactions between WSS females and specific host genes may provide an opportunity to develop pest management strategies to mitigate damage caused by WSS. The integration of knowledge from the fields of plant quantitative genetics, chemical ecology, and insect behavioral ecology allowed insights into the genetic basis of plant intraspecific variations that have important ecological effects on a phytophagous insect species. The approach used here can be broadly applicable to other plant and insect species and may lead to the identification of plant quantitative traits that have important community consequences.

Materials and methods

Insects

Wheat stem sawfly larvae in overwintering diapause were collected from wheat stubble that was heavily infested by WSS in a field near Amsterdam, MT, USA (45°45′29.85″N, 111°22′49.32″W). Larvae were maintained in cold storage (0–4 °C) for 3–6 months to facilitate the completion of diapause. The overwintering 'stubs' were later transferred to plastic tupperware boxes (70 by 35 by 20 cm) and held at room temperature (22–27 °C) for 4–5 weeks until adult emergence. Newly emerged adults were held in 2-L Mason glass jars until experiments were conducted. Narrow wooden skewers were placed inside the jars to allow the insects to perch, rest and climb. Adults used in this study were 24–48-h old and were provisioned with water ad libitum before experimentation.

Near-isogenic line (NIL) development

The NILs used in this study were developed using recombinant inbred line (RIL) populations derived from crosses between the spring wheat lines Reeder/Conan, 'Scholar' (PI 607557)/Conan, and 'Choteau' (PI633974)/Conan. Reeder is a hollow-stemmed line attractive to WSS females (Weaver et al. 2009). Scholar and Conan are both semi-solid-stemmed lines, but Scholar is susceptible to WSS infestation while Conan shows resistance (Talbert et al. 2014). Choteau is widely grown due to stems with a high degree of solidness providing WSS resistance. Data regarding WSS-related traits for the Reeder/Conan and Scholar/Conan RIL populations have been previously reported (Sherman et al.2010; Talbert et al. 2014). The RIL population for Choteau/Conan was not tested in the field as its derivation was solely for the purpose of deriving confirmatory NILs for the present study. Polymerase chain reaction was conducted using microsatellite primer sets to identify NILs for each of the three QTLs. Primer sets included wmc161, linked to the 4A QTL *(Qwss.msub-4A.1)*, identified by Sherman et al. (2010). The marker gwm539 was used for the 2D QTL *Qwss.msub-2D* (Sherman et al. 2010), and gwm340 was used for *Qss.msub-3BL* (Cook et al. 2004). To reduce the genetic variability among segregating lines, heterogeneous inbred families (HIFs) were used as the source of NILs (Barrero et al. 2015). For the Reeder/Conan and Scholar/Conan populations, DNA from a bulk of eight F_8 plants that traced back to a single F_5 plant, developed by single-seed descent from the F_2 generation, was screened with the microsatellite markers. Samples with both alleles were identified. This indicated that the F_5 source plant was heterozygous at the SSR locus. For the bulk F_8 samples that showed both alleles, individual F_8 plants represented in the bulks were re-screened to identify individuals homozygous for each of the alleles. These plants were the seed source for the NIL in these experiments. This is based on the procedure of Pumphrey et al. (2007). For the Choteau/Conan population, approximately 100 F_2 individuals were advanced to the F_5 generation by single-seed descent. Heterozygous individuals were identified by PCR and self-pollinated. Homozygous progeny for each of the alleles were identified as described by Blake et al. (2011). These individuals were the seed source for the NILs used in this study. A pair of resistant and susceptible NILs derived from heterozygous F_5 RIL are expected to be approximately 97 % identical at loci unlinked to the target QTL. In total, four, three, and four NIL pairs polymorphic for *Qss.msub-3BL*, *Qwss.msub-2D*, and *Qwss.msub-4A.1*, respectively, were tested under controlled conditions, while 12, four, and six NIL pairs for *Qss.msub-3BL*, *Qwss.msub-2D*, and *Qwss.msub-4A.1*, respectively, were tested under field conditions. The NILs polymorphic for the *Qwss.msub-2D* were all derived from the cross of susceptible Reeder and resistant Conan, because the other parental lines shared the same marker alleles on the 2D locus. The QTL alleles designated 'a' for *Qwss.msub-2D* and *Qwss.msub-4A.1* indicate alleles previously associated with preference of the WSS for oviposition, while alleles designated 'b' were associated with decreased preference (Sherman et al. 2010; Talbert et al. 2014; Varella et al. 2015). The QTL allele designated 'a' for *Qss.msub-3BL* is the allele for hollow stems from Reeder, the allele designated 'b' is the allele for solid stems from Choteau and Scholar (Cook et al. 2004), and the allele designated 'c' is the solid stem allele derived from Conan (Talbert et al. 2014).

Plant culture

Experiments to monitor WSS oviposition behavior using the NIL pairs were performed in a greenhouse at the Montana State University Plant Growth Center (MSU-PGC, Bozeman, MT, USA). Three plants per pot were grown in tapered square pots (13 by 13 by 13.5 cm) under natural and artificial light (GE Multi-Vapor Lamps model MVR1000/C/U, GE Lighting, General Electric Company, Cleveland, OH), photoperiod of 14:10 (L:D) h, 22 ± 2 °C, and 20–40 % RH. Experiments were conducted from May through August in 2013 and in 2014. Plants were watered regularly and fertilized twice each week with Peters General Purpose Fertilizer (J.R. Peters, Allentown, PA) at 100 ppm in aqueous solution. Soil used 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 Dolomitic lime; Sun Gro Horticulture, Bellevue, WA, USA) (Piesik et al. 2006). All plants used in choice tests were at Zadok's growth stage 32–33 with two to three nodes detectable (Zadoks et al. 1974).

Host preference test

To test the effect of QTLs on WSS host plant selection for oviposition, two sets of cage trials were conducted. In the summer of 2013, choice tests for each pair of NIL and the associated parental lines were conducted with four replications (cages). Each screened cage (91.4 by 66.7 by 91.4 cm with 530 µm mesh openings) received two pots that could be either paired resistant and susceptible NILs or paired parental lines. At 0900 h in day one of the experiment, 15 WSS females and five males were released inside each cage and oviposition was allowed for two consecutive days. Oviposition behavior was observed each day from 1000 to 1300 h, when WSS adults are most active. The duration of events, including females walking on the leaves and walking on stems, was recorded as mean time per female. The number of ovipositor insertions in the stem was recorded. At the end of day two of the experiment, pots were removed from cages, and stems were dissected to assess the number of eggs laid. In the summer of 2014, cage trials were modified to allow the quantification of the number of times females inserted the ovipositor into the stem before rejecting or selecting a plant as an oviposition site. Choice tests for each pair of NIL and the associated parental lines were conducted with 12 replications (cages). Each cage contained two pots that could be paired resistant and susceptible NILs or paired parental lines. At 1000 h of each test day, 15 WSS females and five males were released inside each cage and oviposition was allowed for 2 consecutive hours. Oviposition behavior was observed and the sequence of events that led to an oviposition, including ovipositor insertions on the stem, was recorded. A plant diagram was used to mark the approximate location (stem height in cm) of ovipositor insertions displayed by each female. Immediately after the behavioral observations, pots were removed from cages, and stems were dissected to assess the number and the location (stem height in cm) of eggs deposited. The locations of eggs in the stem were compared to the location where ovipositor insertions were made using the plant diagram. Using the location of the egg on the stem as a starting point, it was possible to trace back the sequence of events that had led to that oviposition. This procedure allowed for the quantification of the number of times females inserted the ovipositor into stems before selection of a plant as an oviposition site. Sequences of events that did not yield eggs were used to quantify the number of times females inserted the ovipositor into the stem before rejecting the plant as an oviposition site.

Y-tube olfactometer

A closed-system Y-tube olfactometer similar to that described by Piesik et al. (2008) was used to test whether QTLs had an effect on behaviorally active volatiles released by wheat plants. Humidified air was fed through a purifying charcoal filter and then split into two air streams using a threaded 24/410 (inner diameter 24 mm) cap with a Teflon liner coupled to a 0.64-cm Swagelok union to deliver air to a pair of glass chambers (40 mm diameter × 800 mm long). Each glass chamber enclosed a single wheat plant as the odor source. Plants were illuminated using an enhanced spectrum LED grow light (Sunshine Systems Grow UFO Light SS-Gu90w) to provide light intensity comparable to field conditions. A flexible Teflon sleeve was tape-sealed around the base of the plant's stems to prevent unfiltered air from entering the system. Teflon tubing then delivered air from the odor source chambers to each arm of the Y-tube. A male ground-glass joint on the stimulus-delivery tube was connected to a female ground-glass joint on each arm of the Y-tube, yielding a consistent airtight fit. A flowmeter was used to set the airflow at 0.1 L/min. Tests were conducted in a 28-mm diameter × 300-mm long Corning glass tubing that branched at 20 cm and had an interior angle of the "Y" of 120°. Diverging arms extended for 4 cm in each direction before becoming parallel for the final 10 cm. The olfactometer was illuminated with a fiber optic illuminator (T-Q/FOI-1, TechniQuip Corp, El Segundo, CA 90245, USA), centered between the Y-tube arms and 30 cm upwind of the bifurcation. This enhanced insect movement as WSS have strong positive phototaxis (Buteler et al. 2009). Paired resistant and susceptible NILs or paired parental lines were used as test stimuli for each bioassay. Females were individually placed at the basal unbranched section of the Y-tube at approximately 2 cm from the outlet. To facilitate insect movement towards the junction of the 'Y', it was necessary to place a long wire in the bottom of the Y-tube, extending 10 cm from the introduction point. Each female was observed for 5 min or until they occupied one of the two arms of the olfactometer. Between 31 and 50 females were used to test each NIL pair, and in successive testing, the stimulus from resistant or susceptible NILs was alternately offered in a different arm of the Y-tube. In total, two pairs of the 2D and 3B NILs, and three pairs of the 4A NILs were tested. All bioassays were conducted at room temperature.

Wheat stem sawfly infestation under field condition

To test the effect of the QTLs on WSS infestation under natural field conditions, the NILs were planted in late April 2014 at a traditionally WSS-infested site near

Loma, MT, USA (48°04′21.96″N, 110°27′41.84″W) and in 2014 and 2015 at a second site with a history of WSS infestation near Amsterdam, MT, USA (45°45′29.85″N, 111°22′49.32″W). Experiments were established adjacent to stubble from a previous WSS-infested wheat crop in a randomized complete block design with two replications in 2014 and 2015. Each block included 12, 4, and 6 NIL pairs for *Qss.msub-3BL*, *Qwss.msub-2D*, and *Qwss.msub-4A.1*, respectively, along with parental lines. Plots were fertilized prior to planting with N, P_2O_5 and K_2O at rates of 112.0, 22.4, and 11.2 kg/ha, respectively. Herbicide (Bromac®, Loveland Products Inc.) was applied in early June at a rate of 1.9 l/ha at the Loma site. Hand weeding was conducted at the Amsterdam sites. Plots consisted of 10 seeds per entry planted in individual hills with spacing of 0.8 m between adjacent hills. Stems were collected at maturity and dissected to determine the presence of WSS larvae (infestation).

Statistical analysis

Results from host preference and host attraction bioassays were analyzed using a Chi-squared test for small sample sizes (Sokal and Rohlf 1995). Data on the duration of specific female behaviors were analyzed using mixed model analysis of variance with maximum likelihood estimation using PROC MIXED in SAS v 9.3 (SAS Institute Inc. 2012). Fixed effects included allele, day, and the interaction, and cage within allele was a random effect. Data were transformed before analysis using a Box–Cox transformation (Box and Cox 1964), but untransformed data are presented. Field data on WSS infestation were analyzed using PROC MIXED in SAS. The model included environment, allele type, and their interaction as fixed effects and replication within environment, family within allele, and interaction of environment and family within allele as random effects.

Results

Host preference for oviposition and oviposition behavior

The number of eggs deposited in stems of the resistant parental line (Conan) was significantly lower than that deposited in stems of the two susceptible parental lines (Reeder and Scholar) ($P < 0.05$) (Table 1). Significant differences between these parental lines were also observed for the number of ovipositor insertions on the stem ($P < 0.05$). Females that had alighted on stems inserted their ovipositor in stems of the susceptible lines (Reeder and Scholar) at more than double the frequency for stems of the resistant line (Conan) (Table 1). Ovipositor insertions into stems of the resistant line (Conan) often did not result in egg deposition (Table 2). Females spent more time ($P < 0.05$) exploring the stem of the more susceptible parent, Reeder, by walking up and down its length (mean ± SE of walk time/per female 20.59 ± 2.31 s; *n* of events = 37) than they did on the resistant parent (Conan) (mean ± SE of walk time/female 8.92 ± 1.24 s; *n* of events = 14), and host examination while walking on stem was always associated with rapid antennal movements of “tapping” the stem (data not shown) as described in Buteler et al. (2009). There were no differences in the duration of time that females walked on stems of the susceptible line Scholar (mean ± SE of walk time/female 20.04 ± 3.66 s; *n* of events = 22) or the resistant line (Conan) (mean ± SE of walk time/female 7.60 ± 1.99 s; *n* of events = 5). Similarly, no differences between any of the parental lines were observed for the duration of walking on the leaves (data not shown).

Preference tests using pairs of NILs confirmed the effects of *Qss.msub-3BL* on host preference for oviposition and probing (ovipositor insertions on stem). The *Qss.msub-3BLc* (derived from the parental line Conan) at the 3B locus significantly reduced the number of ovipositor insertions and eggs laid in the stem for three of the four *Qss.msub-3BL* NIL pairs ($P < 0.05$) (Table 1). An effect for the number of eggs laid was evident for *Qwss.msub-2D*, with the *Qwss.msub-2Db* (derived from the parental line Conan), reducing oviposition in two of three NIL pairs. The *Qwss.msub-2Db* also reduced probing behavior in one of three NIL pairs for *Qwss.msub-2D*. Probing behavior often did not result in egg deposition on NILs with the *Qwss.msub-2Db* ($P < 0.05$) relative to NIL with *Qwss.msub-2 Da* (Table 2). An effect of the *Qwss.msub-4A.1* on number of ovipositor insertions on the stem was observed for the three NIL pairs derived from the Scholar/Conan cross. One of these NIL pairs, SC-3 (Table 2), also differed at the 3B QTL, and thus, it is not possible to determine whether the 3B or 4A QTL caused the difference. No effect of *Qwss.msub-4A.1* was observed for the NIL pair derived from the Reeder/Conan cross. No preference in egg deposition was observed between *Qwss.msub-4A.1* NIL pairs (Table 1). The cumulative outcomes for individual QTLs on WSS oviposition are shown in Fig. 1. The NIL pairs did not differ for the time females spent walking on the leaf nor for the time they spent walking on the stems (data not shown).

Y-tube olfactometer behavioral assays

These bioassays tested the attractiveness of volatile compounds released by NIL pairs polymorphic for three QTLs associated with WSS oviposition preference. More than 65 % of the females preferred volatiles released by plants of the susceptible parental line Reeder over those from the resistant (Conan) plants ($P < 0.05$), but no difference

Table 1 Summary statistics of WSS eggs and ovipositor insertion on wheat stems of near-isogenic lines

Plant	Allele	Number of eggs				Number of insertions			
		Obs	Exp	χ^2	*P* value	Obs	Exp	χ^2	*P* value
Parental lines									
Reeder		24	13.5	16.333	**<0.001**	25	18.0	5.444	**0.020**
Conan		3	13.5			11	18.0		
Scholar		5	2.5	5.000	**0.025**	16	10.0	7.200	**0.007**
Conan		0	2.5			4	10.0		
NIL pair									
2D QTL (*Qwss.msub-2D*)									
RC-1	*Qwss.msub-2Da*	31	22.0	7.364	**0.007**	37	32.5	1.246	0.264
	Qwss.msub-2Db	13	22.0			28	32.5		
RC-2	*Qwss.msub-2Da*	0	0.5	1.000	0.317	28	19.5	7.410	**0.006**
	Qwss.msub-2Db	1	0.5			11	19.5		
RC-3	*Qwss.msub-2Da*	11	5.5	11.000	**<0.001**	22	18.5	1.324	0.250
	Qwss.msub-2Db	0	5.5			15	18.5		
3B QTL (*Qss.msub-3BL*)									
RC-1	*Qss.msub-3BLa*	16	10.5	5.762	0.016	20	13.0	7.538	0.006
	Qss.msub-3BLc	5	10.5			6	13.0		
SC-1	*Qss.msub-3BLb*	3	4.5	1.000	0.317	6	8.5	1.471	0.225
	Qss.msub-3BLc	6	4.5			11	8.5		
SC-2	*Qss.msub-3BLb*	21	11.5	15.695	<0.001	57	30.5	46.05	<0.001
	Qss.msub-3BLc	2	11.5			4	30.5		
SC-3	*Qss.msub-3BLb*	70	46.5	23.753	<0.001	42	31.5	7.000	0.008
	Qss.msub-3BLc	23	46.5			21	31.5		
4A QTL (*Qwss.msub-4A.1*)									
RC-1	*Qwss.msub-4A.1a*	0	0.5	1.000	0.317	11	11.0	0	1.000
	Qwss.msub-4A.1b	1	0.5			11	11.0		
SC-1	*Qwss.msub-4A.1a*	2	1.0	2.000	0.157	14	8.0	9.00	**0.003**
	Qwss.msub-4A.1b	0	1.0			2	8.0		
SC-2	*Qwss.msub-4A.1a*	19	23.0	1.391	0.238	26	17.5	8.257	**0.004**
	Qwss.msub-4A.1b	27	23.0			9	17.5		
SC-3	*Qwss.msub-4A.1a*	21	11.5	15.695	**<0.001**	57	30.5	46.05	**<0.001**
	Qwss.msub-4A.1b	2	11.5			4	30.5		

Bold value indicates *p* values at the 0.05 level of significance

RC source of NIL pair is Reeder/Conan, *SC* source of NIL pair is Scholar/Conan, *Obs* observed value, *Exp* expected value

in attraction was observed for volatiles released by plants from the susceptible line Scholar and those from the resistant plants ($P > 0.05$) (Table 3). Females were significantly attracted ($P < 0.05$) to volatiles emitted by NIL with the susceptible allele *Qwss.msub-4A.1a* for the two NIL pairs derived from the Scholar/Conan cross (Table 3). Bioassays testing the attraction of one pair of *Qwss.msub-4A.1* derived from a Reeder/Conan cross showed no differences between the NILs ($P > 0.05$). Females showed no preference for volatiles released by NIL pairs polymorphic for the *Qss.msub-3BL* or *Qwss.msub-2D* QTLs ($P > 0.05$) (Table 3).

Near-isogenic lines that were shown to be less attractive to WSS females (designated SC-1-*Qwss.msub-4A.1b* and SC-2-*Qwss.msub-4A.1b*) (Table 3) were tested against pure air to ensure that females were not repelled by plant volatiles. Females preferred volatiles coming from SC-2-*Qwss.msub-4A.1b* over the pure air ($P < 0.05$; $\chi^2 = 8.01$; $df = 1$), but there was no difference in choices between the plant and the pure air for SC-1-*Qwss.msub-4A.1b* ($P > 0.05$; $\chi^2 = 0.51$; $df = 1$). The effect of QTLs on different steps of WSS host selection process is illustrated in Fig. 2. The QTL *Qwss.msub-4A.1* affects the ability of WSS females to recognize a suitable host from a distance and the number of times a female inserts the ovipositor inside the stem, while the *Qwss.msub-2D* and *Qss.msub-3BL* QTLs affect the number of ovipositor insertions and

Table 2 Summary statistics of WSS ovipositor insertion into wheat main stems that did not result in oviposition during cage trials of 2014

Plant	Allele	Number of insertions that did not yield eggs			
		Obs	Exp	χ^2	*P* value
Parental lines					
Reeder		2	9.5	11.842	**<0.001**
Conan		17	9.5		
Scholar		2	5.0	3.600	**0.057**
Conan		8	5.0		
NIL pair					
2D QTL (*Qwss.msub-2D*)					
RC-1	*Qwss.msub-2Da*	0	3.0	6.000	**0.014**
	Qwss.msub-2Db	6	3.0		
RC-2	*Qwss.msub-2Da*	5	14.0	11.571	**<0.001**
	Qwss.msub-2Db	23	14.0		
3B QTL (*Qss.msub-3BL*)					
RC-1	*Qss.msub-3BLa*	5	6.0	0.333	0.564
	Qss.msub-3BLc	7	6.0		
SC-3	*Qss.msub-3BLb*	1	9.5	15.211	**<0.001**
	Qss.msub-3BLc	18	9.5		
4A QTL (*Qwss.msub-4A.1*)					
RC-1	*Qwss.msub-4A.1a*	11	12.5	0.360	0.548
	Qwss.msub-4A.1b	14	12.5		
SC-2	*Qwss.msub-4A.1a*	1	4.0	4.500	**0.034**
	Qwss.msub-4A.1b	7	4.0		

Bioassays were conducted in 2014 using parental lines and near-isogenic lines polymorphic for distinct resistance quantitative trait loci

Bold value indicates *p* values at the 0.05 level of significance

RC source of NIL pair is Reeder/Conan, *SC* source of NIL pair is Scholar/Conan, *Obs* observed value, *Exp* expected value

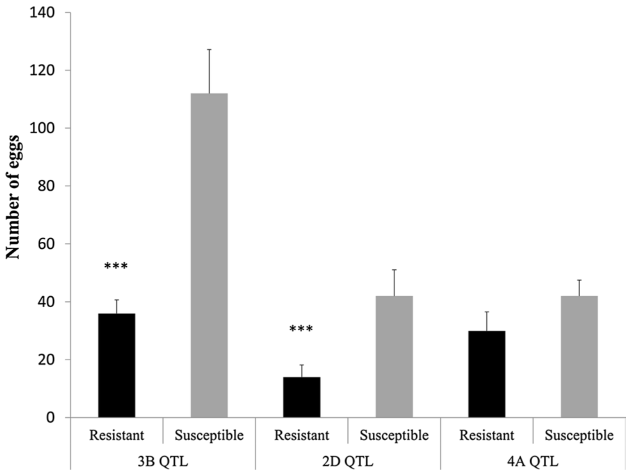

Fig. 1 Wheat stem sawfly preference for oviposition on near-isogenic lines polymorphic for resistance QTLs. Resistance QTLs are located on chromosomes 3B, 2D, and 4A. Number of eggs are combined over all pairs of NILs tested.***Significant differences ($P < 0.001$) based on Chi-squared test analysis. Susceptible alleles are defined as those that lead to increased oviposition by the wheat stem sawfly *(Qwss.msub-2Da*, *Qss.msub-3BLa and Qss.msub-3BLb*, *and Qwss.msub-4A.1a*, respectively), while resistant alleles are defined as those that lead to decreased oviposition (*Qwss.msub-2Db*, *Qss.msub-3BLc*, *and Qwss.msub-4A.1b*, respectively)

egg deposition once the female is in contact with the host. The QTL *Qss.msub-3BL* had the greatest effect (Table 1).

Wheat stem sawfly infestation under field conditions

The susceptible parental lines Reeder and Scholar were significantly ($P > 0.05$) more infested than the resistant Conan (Table 4). No effect on WSS field infestation was observed for NIL pairs for *Qwss.msub-2D*, *Qss.msub-3BL* or *Qwss.msub-4A.1* (Table 4).

Discussion

The oviposition behavior of WSS was first described by Ainslie (1920). In this early description, two main factors were identified as important determinants of oviposition, the plant growth stage and the environmental conditions during host plant selection. Subsequent studies showed that physical (e.g., stem diameter and length) and chemical (e.g., attractive volatiles) characteristics of host plants also influence female choice for oviposition (Holmes and Peterson 1960; Piesik et al. 2008; Weaver et al. 2009). A general functional organization of the WSS oviposition process was presented by Buteler et al. (2009) based on measurements of the transitional frequencies between different behaviors displayed during host plant selection. Briefly, WSS host selection starts with external host evaluation, characterized by walking on leaves and stems, followed by internal host evaluation through ovipositor insertions into the stem, and a final decision of host acceptance or rejection when choosing to oviposit. Our study describes the effect of three host plant QTLs on female choices during oviposition. This was made possible by the development of NILs for specific wheat QTLs impacting WSS resistance. Each NIL pair differed primarily only for the gene of interest at the specific QTL. This allowed the NIL pairs to be tested side-by-side on the same day, avoiding confounding issues such as amount of sunshine on any given day. The latter is especially important for WSS which exhibits strong phototaxis (Buteler et al. 2009).

Results presented herein confirmed that host selection by WSS occurs in a stepwise fashion, with plant cues modulating different behavioral sequences. Allelic

Table 3 Responses of female WSS to volatiles released by wheat near-isogenic lines in a Y-tube olfactometer

Plant		Allele	Behavioral responses			
			Obs	Exp	χ^2	*P* value
Parental lines						
Reeder			28	21.5	3.930	**0.047**
Conan			15	21.5		
Scholar			15	17.5	0.714	0.398
Conan			20	17.5		
NIL pair						
2D QTL (*Qwss.msub-2D*)						
	RC-1	*Qwss.msub-2Da*	27	22.5	1.800	0.179
		Qwss.msub-2Db	18	22.5		
	RC-2	*Qwss.msub-2Da*	16	20	1.600	0.206
		Qwss.msub-2Db	24	20		
3B QTL (*Qss.msub-3BL*)						
	RC-1	*Qss.msub-3BLa*	16	15.5	0.032	0.179
		Qss.msub-3BLc	15	15.5		
	SC-3	*Qss.msub-3BLb*	27	23.5	1.042	0.307
		Qss.msub-3BLc	20	23.5		
4A QTL (*Qwss.msub-4A.1*)						
	RC-1	*Qwss.msub-4A.1a*	22	20	0.400	0.527
		Qwss.msub-4A.1b	18	20		
	SC-1	*Qwss.msub-4A.1a*	31	23.5	4.787	**0.029**
		Qwss.msub-4A.1b	16	23.5		
	SC-2	*Qwss.msub-4A.1a*	35	25	8.000	**0.005**
		Qwss.msub-4A.1b	15	25		

Bold value indicates *p* values at the 0.05 level of significance

RC source of NIL pair is Reeder/Conan, *SC* source of NIL pair is Scholar/Conan, *Obs* observed value, *Exp* expected value

variations in individual host plant QTLs are sufficient to affect female choice and to determine host preference hierarchy. Because at least one of these QTLs is associated with larval mortality (Talbert et al. 2014), female decisions to oviposit suggest an adaptation to optimize progeny performance and survival (Jaenike 1978; Thompson 1988). To our knowledge, this is the first time that allelic variations on a host plant QTL affecting larval survival are shown to also influence female oviposition behavior and host preference.

Qwss.msub-4A.1: a QTL for host plant attractiveness

Host selection by WSS begins before landing, with females evaluating long-distance cues emitted by host plants. One of these cues is at least partially controlled by the *Qwss.msub-4A.1*, which was shown to affect host plant attractiveness to foraging females. Wheat plants have a characteristic 'green' odor due to eight volatile compounds of 6-carbon aldehydes and alcohols (Hatanaka 1993). Two of these compounds, (*Z*)-3-hexenyl acetate and (*Z*)-3-hexen-1-ol, are attractive to foraging WSS females, as is the terpenoid (*E*)-β-ocimene (Piesik et al. 2008). The parental lines Reeder (susceptible) and Conan (resistant) have been shown to differ for the emission of (*Z*)-3-hexenyl acetate, with the resistant line being less attractive and releasing a smaller amount of the attractive compound (Weaver et al. 2009). As pointed out by Weaver et al. (2009), quantitative variation in the release of this chemical signal might explain differences in attraction between these two parental lines. In these experiments, differences in attraction were observed between all the *Qwss.msub-4A.1* NIL pairs derived from the Scholar/Conan cross but not from the one NIL pair derived from Reeder/Conan (Table 3). This indicates that genes other than *Qwss.msub-4A.1* may also play a role in host plant attractiveness to foraging females. It is important to note that bioassays were not designed to quantify the emission of individual volatile compounds. Thus, it is unclear whether or not NILs differed for the emission of (*Z*)-3-hexenyl acetate, (*Z*)-3-hexen-1-ol, (*E*)-β-ocimene or other unknown chemical signals.

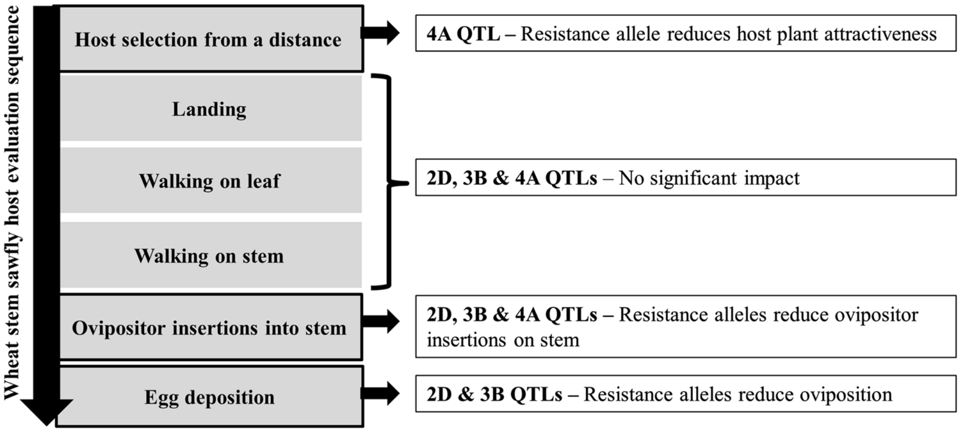

Fig. 2 Wheat QTLs associated with host selection and oviposition behavior by the wheat stem sawfly. Susceptible alleles are defined as those that lead to increased oviposition by the wheat stem sawfly (*Qwss.msub-2Da*, *Qss.msub-3BLa and Qss.msub-3BLb*, *and Qwss.msub-4A.1a*, respectively), while resistant alleles are defined as those that lead to decreased oviposition (*Qwss.msub-2Db*, *Qss.msub-3BLc*, *and Qwss.msub-4A.1b*, respectively)

Table 4 Summary statistics of field infestation of WSS in pairs of near-isogenic lines

Pairing source of NILs	Allele type	Infestation (%)
Parental lines		
Reeder		30.99 **±** 6.96**A**
Scholar		24.50 **±** 7.01**A**
Conan		13.07 **±** 2.88**B**
Choteau		11.05 **±** 3.42**B**
2D QTL (*Qwss.msub-2D*)		
Reeder/Conan	*Qwss.msub-2Db*	15.11 ± 2.34A
	Qwss.msub-2 Da	17.09 ± 2.21A
3B QTL (*Qss.msub-3BL*)		
Reeder/Conan	*Qss.msub-3BLc*	18.99 ± 3.96A
	Qss.msub-3BLa	17.24 ± 3.18A
Scholar/Conan	*Qss.msub-3BLc*	18.89 ± 2.13A
	Qss.msub-3BLb	20.02 ± 2.58A
Choteau/Conan	*Qss.msub-3BLc*	18.43 ± 2.39
	Qss.msub-3BLb	19.47 ± 2.37A
4A QTL (*Qwss.msub-4A.1*)		
Reeder/Conan	*Qwss.msub-4A.1b*	17.93 ± 4.04A
	Qwss.msub-4A.1a	17.95 ± 4.84A
Scholar/Conan	*Qwss.msub-4A.1b*	15.61 ± 2.48A
	Qwss.msub-4A.1a	16.43 ± 2.43A

Mean values with different letters within a pairing source denote significant differences according to t test (LSD) at $P < 0.05$. Mean averaged over three environments, Loma 2014, Amsterdam 2014, and Amsterdam 2015

Bold value indicates p values at the 0.05 level of significance

Qss.msub-3BL and *Qwss.msub-2D*: QTLs for oviposition preference

While in contact with the host plant, female WSS assess external information about the plant by walking on leaves and stems (Buteler et al. 2009), but none of the QTLs affected this stage of the host selection process. Internal information about the host plant is assessed by repeatedly inserting and withdrawing the saw-like ovipositor inside the stems. Similar behavioral displays were also reported for *Cephus pygmaeus* (Hymenoptera: Cephidae) (Ries 1926) and for the gall-inducing sawfly, *Euura lasiolepis* (Hymenoptera: Tenthredinidae) (Roininen et al. 1999). As reported for *E. lasiolepis*, WSS females inserted their ovipositor more often in preferred host plants, yet ovipositor insertions did not necessarily result in egg deposition (Table 2). Thus, plant cues, perhaps including puncture resistance at the stem wall and solidity of the stem, assessed in this step of the host selection process appear to be important determinants of host acceptance. All three QTLs studied here affected probing behavior; so, multiple plant cues are evaluated by WSS females in this specific step of the selection process. Once females examined internal characteristics of the host stem, allelic differences in the *Qws.msub-4A1* locus did not affect the decision of accepting or rejecting a host. However, allelic variations at *Qwss.msub-2D* and *Qss.msub.3BL* influenced female choices for oviposition made when probing (Table 1).

The *Qss.msub-3BL* affected ovipositor insertions and egg deposition in the stem. Low levels of natural WSS infestation during the study years may have precluded the observation of QTL effect for populations of NILs in the field study. Infestation levels in Sherman et al. (2010) and Talbert et al. (2014) which suggested the effect of 3B, 4A (Sherman et al. 2010; Talbert et al. 2014) and 2D (Sherman et al. 2010) QTLs on Reeder/Conan and Scholar/Conan RILs were greater than the ones observed in this study. Previous studies have shown that lines containing the susceptible allele from Reeder have hollow stems (Sherman et al. 2010), while lines containing the *Qss.msub-3BLc* or *Qss.msub-3BLb* alleles have intermediate levels of stem solidness (Talbert et al. 2014). Despite the similarities in the degree of stem solidness conferred by the Conan and Scholar alleles, the *Qss.msub-3BLc* allele causes higher levels of larval mortality (Talbert et al. 2014). Interestingly, NILs with the *Qss.msub-3BLc* allele were also significantly less preferred than NILs containing either the *Qss.msub-3BLa* or the *Qss.msub-3BLb* alleles. Taken together, these results suggest that WSS host selection appear to be in accordance with the preference–performance hypothesis, which postulates that females preferences for oviposition are adapted to optimize progeny performance (Jaenike 1978; Thompson 1988). Furthermore, *Qss.msub-3BL* seems to determine the proximate mechanistic explanations for both larval mortality and the ability of females to detect suitable stems for oviposition. Ultimately, it appears that these decisions are linked to the overall favorability of the host, because multiple females following the behavioral sequence outlined in Buteler et al. (2009) can select the same stem even if it is already infested with WSS eggs or feeding larvae. However, this experiment (Buteler et al. 2009) evaluated oviposition decisions among stems from a single susceptible line of wheat. Subsequent oviposition events have increased internecine risk for the offspring, but assessment of host suitability is decisive, with previous infestation having no influence on the outcome.

An issue that may have affected WSS reaction to the NIL pairs was background alleles at the other QTLs. In one case, females exposed to the *Qwss.msub-2D* NIL pair RC-2 (Table 1) displayed a different behavioral response than those exposed to other *Qwss.msub-2D* NIL pairs. The pair RC-2–*Qwss.msub-2D* had their stems intensively probed by females, but only a single egg was laid (Table 1). This NIL pair had a *Qwss.msub-4A.1a* allele at the 4A locus, which would have made both plants attractive to females;

but they also had a *Qss.msub-3BLc* allele at the 3B locus, which would have made both plants less preferred for oviposition. In general, NIL pairs that had the allele derived from the parental line Conan at the *Qss.msub-3BL* (e.g., RC-2-*Qwss.msub-2D*, SC-1-*Qwss.msub-4A.1.1*) (Table 1) tended to have very few eggs. This might have precluded detection of differences between NILs with different alleles at either *Qwss.msub-2D* or *Qwss.msub-4A.1*. These observations suggest that plant cues might be processed by females synergistically, with the effect of different cues combining to determine host suitability for oviposition.

Host preference for oviposition explored in this study is relative rather than absolute. This has important implications for the utilization of antixenosis QTLs for WSS management. Females could potentially lay more eggs than would be expected on resistant plants, provided more suitable hosts were rare or not available. Thus, in wheat monoculture, the use of less preferable/attractive varieties might not have the desirable effect on pest management. Varieties with antixenotic properties may be better employed in the context of trap cropping of WSS (Morrill et al. 2001). This is a behavioral manipulation method that reduces pest abundance via the integration of a push–pull strategy with a subsequent pest mortality caused either by trap crop destruction or antibiosis mechanisms of resistance (Cook et al. 2007). Marker-assisted selection would be a key tool for the development of such varieties and the implementation of push–pull strategies in wheat fields.

Conclusions

This study provides repeated evidence that host discrimination by phytophagous insects can occur in a fine scale, with ovipositing females being able to discriminate variation in plant cues resulting from allelic variations within individual plant QTLs. In the case of WSS, maternal choices during oviposition appear to reflect adaptation to optimize progeny performance and survival. This study represents a step toward understanding the genetic mechanisms modulating the complex process of host selection by WSS. From a practical perspective, QTLs shown to affect WSS oviposition preference and host attraction may provide a complement to the current use of solid stems as an antibiosis mechanism to improve pest management in wheat fields.

Author contribution statement DKW, LT, and ACV conceived and designed the experiments. JDS, NKB, LT, and ACV developed near-isogenic lines. DKW and MLH provided insects for experiments. ACV performed the experiments. ACV and JMM analyzed the data. ACV, DKW, TL, and RKDP wrote the manuscript. All authors critically read, commented, corrected and approved the final manuscript.

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Compliance with ethical standards

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

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