DOI: 10.1111/j.1570-7458.2007.00550.x

Oviposition behavior of orange wheat blossom midge on low- vs. high-ranked grass seed heads

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Accepted: 25 January 2007

Key words: Sitodiplosis mosellana, Diptera, Cecidomyiidae, *Triticum aestivum*, *Hordeum vulgare*, host selection, examining, host-plant resistance

Abstract

The discovery of *Sm1*, a highly effective resistance (*R*) gene that targets the first instar of the orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), has created concerns about wheat midge adaptation. Strategies for delaying adaptation to *Sm1* include the simultaneous deployment of a resistance trait targeting a different life stage, i.e., the ovipositing adult female. Previous studies have shown that adult females distinguish between wheat genotypes and seed head developmental stages and are attracted by volatiles from young wheat heads. We focused on what happens after the female lands on the seed head, comparing in three tests a seed head of the high-ranked pre-anthesis 'Roblin' wheat, *Triticum aestivum* L. (Poaceae), and a head of one of three lower-ranked types: post-anthesis 'Roblin', pre-anthesis 'Key' wheat (*T. aestivum*), and pre-anthesis 'Robust' barley (*Hordeum vulgare* L.). Within each test, high- and low-ranked heads were presented in choice and no-choice assays, with the behavior of wheat midge females scored every 5 min from 20:30 to 23:00 hours, under mid-summer natural light conditions and sunset occurring between 20:50 and 21:20 hours. Head type influenced both proportions of females observed on the head and proportions of females probing with the ovipositor. Head*assay interactions occurred only in the test comparing wheat to barley, with barley reducing females observed on the wheat head and wheat increasing females probing on barley. Results indicate that the wheat midge female detects plant cues while examining the seed head and that this detection contributes to differences in egg counts.

Introduction

The revolution in molecular biology is changing ideas about which plant traits might be manipulated in the future and how novel resistance traits could be combined (Ferry et al., 2004). For example, it may be possible to combine, in a single cultivar or in two cultivars grown side by side, plant traits that affect the performance of juvenile feeding stages with plant traits that influence host finding and selection by adult females (Gould, 1986). Combining two plant resistance traits that target different life stages is expected to slow the rate at which the pest adapts to individual plant resistance traits (Gould, 1986; Rausher, 2001).

Combining plant traits targeted at juveniles with plant traits targeted at adults has been proposed for the orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) (Lamb et al., 2002; Smith et al., 2004). The orange wheat blossom midge (hereafter referred to as wheat midge) attacks developing grass seeds and is a serious pest of wheat in North America (Olfert et al., 1985; Lamb et al., 1999), Europe (Oakley et al., 1998), and China (Ni & Ding, 1994). Effective host-plant resistance to wheat midge has been sought for many years. In 1996, the first highly effective resistance trait was reported (Barker & McKenzie, 1996). Subsequently, this trait was shown to be associated with a single partially dominant resistance (*R*) gene called *Sm1* (McKenzie et al., 2002). *Sm1* reduces survival of first-instar wheat midges by 99% (Lamb et al., 2000). If wheat genotypes carrying *Sm1* perform well in Canadian registration trials, deployment will begin within 1–5 years in the hard red spring wheat (*Triticum aestivum* L.) (Poaceae) region of the northern Great Plains (Berzonsky et al., 2003). Deployment of the same gene in durum wheat (*Triticum turgidum* L.) is expected to follow.

The wheat midge is expected to adapt to *Sm1* (Lamb et al., 2000; McKenzie et al., 2002). Two cecidomyiid relatives of the wheat midge, Hessian fly, *Mayetiola destructor* (Say), and rice gall midge, *Orseolia oryzae* (Wood-Mason), have

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adapted to similar resistance genes targeting first instars, exhibiting virulence as little as 3 years after the *R* gene was deployed (Gould, 1998; Sardesai et al., 2001; Harris et al., 2003). On plants with the 'defeated' resistance gene, the crop again suffers losses while adapted virulent larvae survive with no apparent loss of fitness. Fortunately for these two pest species, other *R* genes are available to replace the 'defeated' *R* gene, i.e., >30 *R* genes for Hessian fly and >6 for rice gall midge. This is not the case for wheat midge, which has only the one documented *R* gene. One strategy for delaying the adaptation of wheat midge is to combine *Sm1* with a plant trait that interferes with host finding and selection by the adult female (Lamb et al., 2001; Berzonsky et al., 2003; Smith et al., 2004).

Unfortunately, little is known about adult female behavior. In a four-arm olfactometer, females were attracted by volatile chemicals from pre-anthesis wheat heads (Birkett et al., 2004). Most commonly, wheat midge eggs are oviposited on the inner concealed surfaces of two modified leaves that protect the developing seed, i.e., the glume and lemma (Smith & Lamb, 2001). Based on egg counts, females distinguished between wheat (*T. aestivum* L.) genotypes: 'Roblin' > 'Superb' = 'Key' (Lamb et al., 2003). Pre-anthesis wheat seed heads received nine times more eggs than post-anthesis heads (Ding & Lamb, 1999). Low-ranked head types consistently received fewer eggs in field and laboratory tests but this was not associated with any morphological trait of the seed head, e.g., the presence of awns or the length, width, hairiness, toughness, or waxiness of the modified leaves (Lamb et al., 2001; Smith & Lamb, 2001). Apart from *T. aestivum* and *T. turgidum*, the wheat midge oviposits and survives on 15 other *Triticum* species (Wise et al., 2001), as well as on rye (*Secale* L.), barley (*Hordeum* L.), and a number of wild grasses (Barnes, 1956; Wright & Doane, 1987; Harris et al., 2003).

Our ultimate research aim is to identify plant traits that influence the behavior of wheat midge females. We began our studies by addressing the following questions: Can behavior be observed and quantified, in spite of the female's small size (body length: 3 mm), pale color, and nocturnal habits? Do females behave differently in response to low-ranked vs. high-ranked seed heads? Do behavioral responses to low- and high-ranked seed heads differ in choice vs. no-choice assays?

Materials and methods

Insects and plants

Adult wheat midges either emerged directly from 2003 field-collected third instars (from common and durum wheat in Mohall, ND, USA, at 48°76′N, 101°51′W) or a wheat midge colony collected in 2002 (from durum wheat in Wildrose, ND, USA, at 48°59′N, 103°21′W) and reared in the greenhouse on 'Roblin' hard red spring wheat for one generation. For greenhouse-reared insects, pre-anthesis wheat heads were exposed to adult females either individually (in a 5 × 12 cm in diameter cylindrical glass cage with netting on either end and closed with a clip) or as a group (in a fine-netted bag over 10–20 wheat heads). Adult females (5–15) were introduced into each cage at 18:30 hours using an aspirator. One to 3 days later, cages were removed and heads were covered with 5 × 18 cm glassine pollinating bags (Lawson Pollinating Bags Inc, Northfield, IL, USA) to enhance larval survival during eclosion and migration to feeding sites. Three to 4 weeks later, stems were cut and heads were brought to the laboratory. Wheat heads containing mature larvae were first air dried for several weeks and then were broken apart to extract larvae. Wheat fragments were shaken through a sieve (No. 12, USA Standard testing sieve, 1.7 mm opening, W.S. Tyler Inc, Mentor, OH, USA) held over a white enamel tray. From the sieved wheat fragments, the larvae, which are orange, were collected with a camel hair brush and placed in lidded plastic containers containing a layer of sterile moistened silica sand (1 cm) on top of a layer (4 cm) of potting soil. Containers were left at room temperature (20 ± 3 °C) until all larvae had entered the soil (2–3 weeks) and formed over-wintering cocoons. Containers then were transferred to a cold room (2.5 ± 2.0 °C) where they remained for a minimum of 4 months and a maximum of 12 months.

When adults were needed, containers were removed from the cold and placed in cages held in an environmental chamber with artificial lighting (20 ± 4 °C, 60 ± 20% r.h., and L16:D8). Cages consisted of plastic boxes (35 × 23 at the base × 27 cm in height) with a fine mesh ceiling and 3 cm of moist soil lining the bottom. Adults eclosed 3–7 weeks later. Males started emerging several days earlier than females and also emerged earlier in the day, i.e., in the early to mid-afternoon vs. the late-afternoon for females (Pivnick & Labbé, 1992). Given high humidity conditions and moderate temperatures (ca. 20 °C) adult females live for about 4–5 days. For choice tests and behavioral observations, only 2-day-old females were used. Wheat midge females carry 60–80 eggs (Pivnick & Labbé, 1993). Oviposition begins on the second night following eclosion (1-day-old females) and females oviposit the greatest number of eggs on the third night (2-day-old females). Prior to testing, females had been held since eclosion with males but had not been given access to plant material.

Three grass genotypes were used in tests: two hard red spring wheat (*T. aestivum*) cultivars, 'Roblin' and 'Key 97–24', and one six-rowed malting barley (*H. vulgare*) cultivar, 'Robust'. 'Key' seed was provided by the Cereal Research

Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba, Canada. This cultivar was chosen because it averages 65% fewer eggs relative to stimulatory wheat such as 'AC Barrie' and 'Roblin' (Smith et al., 2004). For all three genotypes, heads were either at the pre-anthesis stage (hereafter referred to as 'young' heads; head emerged but anthers not yet beginning to show Zadoks stage 58–59) (Zadoks et al., 1974) or ca. 6 days post-anthesis (hereafter referred to 'old' heads; pollen already shed and anthers white). Within a single night's tests (i.e., a block), all test plants were either grown indoors or outdoors. Starting in April 2004, indoor plants were grown in an environmental chamber (20 °C, 590–710 μmol per m^2 per s light intensities, L16:D8, Conviron®, E-8 model, Winnipeg, Manitoba, Canada). Starting in May 2004, outdoor plants were grown under variable conditions (5–25 °C, L18:D6 to L19:D5) inside a $3 \times 2 \times 2$ m cage, consisting of a wooden frame and insect-proof mesh cloth. For plants grown singly, a seed was planted in a plastic cone (25 cm high × 6.4 cm in diameter) (Cone-tainer, Stuewe and Sons, Inc., Corvallis, OR, USA). For plants grown in groups, 9–25 seeds were planted in a plastic pot (25 cm in height × 30 cm in diameter; Stuewe and Sons, Inc.). At planting, a slow-release fertilizer (Scotts®, 15 : 9 : 12 N : P : K; Scott's Sierra Horticultural Product Company, Maysville, OH, USA) was added to a standard potting soil (Sunshine® SP 100, Sun Gro Horticulture Inc., Bellview, WA, USA). Plants were watered daily.

All tests were conducted in the greenhouse under natural light conditions during a 6-week period starting in late-June of 2004. Sunset initially occurred at 21:20 hours, but during the 6-week test period occurred earlier and earlier, with sunset finally at 20:50 hours. Excised grass heads were used in all tests except the test comparing excised to non-excised heads. The procedure for obtaining excised heads was from Lamb et al. (2003). Two hours before the test began, a wheat stem was submerged in water and was cut 20 cm below the distal end of the seed head. The stem was then moved (while still under water) to a water-filled 7-cm plastic floral tube (Floral Pik, Dakota Plastics, Watertown, SD, USA). Non-excised grass heads were handled in the same manner except that stems were not cut. A microscope was used to count eggs. The modified leaves (i.e., the glume, lemma, and palea) comprising each spikelet of the seed head were removed, with all surfaces examined for eggs. Subsequently, the rachis was examined. The precise location of eggs within the head was not recorded.

Egg-count tests to develop methodology

We first tested whether female wheat midge oviposit similar numbers of eggs on excised and non-excised seed heads. Four young 'Roblin' heads of a similar size and maturity were selected. Two excised heads (held in place by taping the floral tube to a bamboo pole) and two non-excised heads were placed at a uniform height in a 2×2 formation (15 cm between heads in adjacent corners). The two poles holding the floral tubes with an excised head and the two cones holding the unexcised heads were placed vertically in a 30-cm diameter pot, using a 25-cm layer of moist potting mix in the pot to stabilize the poles and cones. A metal frame (three circles of increasing size held in place by four vertical straight wires) was placed over the pot. Netted material was placed over the metal frame and was positioned ca. 15 cm away from the four wheat heads (above and sides) and was secured by clips to the pot and frame. Excised heads were placed in floral tubes at 18:00 hours. The test was set up by 18:30 hours, when females were aspirated into cages, and ended at 09:00 hours when eggs were counted. On each of five nights (blocks), two choice tests (20 females per cage) were conducted (10 replicates). Analysis of variance (ANOVA) tested for effects of head type and block.

In the second test, excised heads of four types were presented to females in a choice test. Four tubes containing one old head of 'Roblin' or one of three young heads, 'Roblin' wheat, 'Key' wheat, or 'Robust' barley, were placed at a uniform height in a 2×2 formation (8-cm spacing between heads) by positioning tubes in moist soil held in a 15-cm diameter plastic pot. A cylindrical plastic cage (25 cm in height × 15 cm in diameter, fine mesh on top) was then placed over the four heads. Excised heads were placed in floral tubes at 18:00 hours. At 18:30 hours, 10 2-day-old females were aspirated into the arena. The next morning at 10:00 hours, heads were removed and eggs were counted. Fifteen replicates were completed, three on each of five nights (blocks). ANOVA tested for effects of head type and block.

The third test compared eggs/head for a cage that contained four females vs. a cage that contained four females + four males. A floral tube holding an excised wheat head was positioned in the middle of a sand-filled clear plastic cup (diameter 9 cm × height 12 cm) and was covered with an inverted clear plastic cup (12 cm in height × 10 cm in diameter, with fine mesh ceiling). Excised heads were placed in floral tubes at 18:00 hours. The test was set up by 18:30 hours and ended the next day at 09:00 hours, when eggs were counted. Fourteen replicates were completed, 2–3 per night over six nights (blocks). ANOVA tested for effects of males and block.

In the fourth test, we used a factorial design testing for two time periods, 20:00–24:00 hours vs. 24:00–08:00 hours, and two head types, young vs. old 'Roblin' heads. Each cage (same as those described for the third test) contained a single floral tube with its excised head. At 20:00 hours, five females were introduced into each cage. At 24:00 hours,

this first head was removed and replaced with a second head of the same type. If a female was on a head at the time of removal, the head was gently moved until the female flew away. At 08:00 hours, the second head was removed and all heads were checked for eggs. Six replicates were completed, 1–2 per night over four nights (blocks). ANOVA tested for effects of head type, time, and block, as well as head*time interactions.

Behavioral observations

The recording rule defines how behavior is recorded (Martin & Bateson, 1993). We selected instantaneous sampling as our recording rule, dividing our observation session (20:30–23:00 hours) into 5-min intervals. This relatively long sample interval was chosen to avoid observer fatigue (which compromises reliability of measurement) when quickly scoring, at each sample interval, three categories of behavior occurring on six different heads.

The sampling rule specifies which individuals will be watched and when (Martin & Bateson, 1993). Here we had planned to use focal animal sampling and a series of cages, each of which contained a single female. This plan was reassessed after initial tests in which individual females given the high-ranked young 'Roblin' exhibited extremely low levels of activity (see Results). To test if females would be more active if held in groups, we used a factorial design to compare two female densities (one female per cage vs. four females per cage), and two head types (young vs. old 'Roblin' heads). At 18:00 hours, 10 glass cages (17 cm in height × 4 cm in diameter, transparent glass wall with fine mesh ceiling) were arranged in a north–south line (with young and old heads alternated), with a clear view of the western sky behind the cages and no artificial lighting. In each cage, there was a single young or old 'Roblin' head (alternating within the line), with each head arranged at a uniform height (2 cm below the cage ceiling). At 20:28 hours, two of the cages, one with a young head and one with an old head, each received four females. The other eight cages, four with young heads and four with old heads, each received a single female. Starting at 20:30 hours, each cage was scan sampled for numbers of females on the head. Scan sampling of all 10 cages was completed within 1 min. Thirty minutes after sunset, when it became more difficult to see the insects, sampling was achieved by focusing a flashlight with a red filter on the head for the 3–6 s it took to score females.

Because grouped females exhibited adequate levels of activity, the three subsequent tests were conducted using scan sampling as the sampling rule and instantaneous sampling as the recording rule. Each test compared behavior on two head types: (i) young 'Roblin' vs. old 'Roblin', (ii) young 'Roblin' vs. young 'Key', and (iii) young 'Roblin' vs. young 'Robust' barley. At 18:00 hours, three heads of each of the two types were excised, placed in floral tubes, and arranged uniformly within the cage (3 cm between two heads and between head and closest cage wall; 2 cm from top of the head to the cage ceiling). The cage was a clear plastic cylinder with a fine mesh ceiling (17 cm in height × 9 cm in diameter). In one of the three cages, the two head types were present (choice), while in the other two cages only a single head type was present (no-choice). At 20:28 hours, four females were introduced into each cage. Scan sampling began at 20:30 hours and continued at intervals of 5 min until 23:00 hours, when heads were removed from cages and held for egg counts the following day. For each cage, we recorded the number of females exhibiting any of three behaviors that occurred on the seed head: (i) probing with the tip of the ovipositor contacting head surfaces, (ii) sitting with the ovipositor telescoped into the abdomen, and (iii) walking without the ovipositor contacting head surfaces. For each experiment, scan sampling was conducted on 6 nights (replicates). Rather than completing a single test and then moving on to the next test, replicates of the three tests were alternated over a period of 3 weeks (July 2004).

The proportion of females on each seed head was calculated as a proportion of the total females that could possibly have been on the head throughout the observation period, i.e., 124 females per cage (31 intervals × four females per cage). Thus, numbers of females actually observed on each head were totaled for all 31 intervals and divided by 124 females per cage. The proportion of females exhibiting a specific behavior (probing, sitting, or walking) was calculated as a proportion of the total females observed on the head. Thus, numbers of females observed displaying the behavior were totaled for all 31 intervals and divided by the total number of females observed on the head over the 31 intervals.

Statistical analyses

Statistical analyses were performed using JMP 5.01 (SAS Institute Inc, 2002). Data were first tested for homogeneity of variance (O'Brien's test, $P<0.05$). When variances were homogeneous, we proceeded with the ANOVA. When variances were heteroscedastic, an attempt was made to rectify the problem with data transformation. If the problem persisted, a Welch ANOVA was used. When treatment effects were significant ($P<0.05$), the Tukey–Kramer honestly significant difference test was used to separate means ($P<0.05$).

Results

Egg-count tests to develop methodology

In the first test, egg counts on excised and non-excised young 'Roblin' wheat heads were similar (ANOVA,

Table 1 For each paired comparison of a high- vs. low-ranked head type, effects of head type and assay type (choice vs. no-choice)

Head types compared	Effects in model	Percentage on head[1]	percentage probing[1]	percentage sitting[1]	percentage walking[1]	Number of eggs per head[1]
Test 1						
Young 'Roblin' wheat	Head	$F_{1,27} = 44.45^{***}$	$F_{1,27} = 17.36^{**}$	$F_{1,27} = 10.80^{**}$	$F_{1,27} = 4.12$ ns	$F_{1,27} = 96.67^{***}$
vs.	Assay	$F_{1,27} = 0.06$ ns	$F_{1,27} = 0.03$ ns	$F_{1,27} = 0.42$ ns	$F_{1,27} = 1.11$ ns	$F_{1,27} = 1.11$ ns
old 'Roblin' wheat	Head*assay	$F_{1,27} = 0.23$ ns	$F_{1,27} = 0.25$ ns	$F_{1,27} = 1.64$ ns	$F_{1,27} = 0.78$ ns	$F_{1,27} = 0.00$ ns
	Block	$F_{5,27} = 4.10^{**}$	$F_{5,27} = 2.69^{*}$	$F_{5,27} = 3.30^{**}$	$F_{5,27} = 1.15$ ns	$F_{5,27} = 3.18^{*}$
Test 2						
Young 'Roblin' wheat	Head	$F_{1,27} = 36.64^{***}$	$F_{1,27} = 33.47^{***}$	$F_{1,27} = 9.83^{**}$	$F_{1,27} = 6.06^{**}$	$F_{1,27} = 57.03^{***}$
vs.	Assay	$F_{1,27} = 1.58$ ns	$F_{1,27} = 0.74$ ns	$F_{1,27} = 1.23$ ns	$F_{1,27} = 0.19$ ns	$F_{1,27} = 0.01$ ns
young 'Key' wheat	Head*assay	$F_{1,27} = 1.25$ ns	$F_{1,27} = 1.36$ ns	$F_{1,27} = 0.14$ ns	$F_{1,27} = 0.69$ ns	$F_{1,27} = 0.62$ ns
	Block	$F_{5,27} = 1.42$ ns	$F_{5,27} = 1.83$ ns	$F_{5,27} = 2.08$ ns	$F_{5,27} = 0.64$ ns	$F_{5,27} = 0.95$ ns
Test 3						
Young 'Roblin' wheat	Head	$F_{1,27} = 143.56^{***}$	$F_{1,27} = 245.79^{***}$	$F_{1,27} = 28.23^{***}$	$F_{1,27} = 5.69^{**}$	$F_{1,27} = 101.24^{***}$
vs.	Assay	$F_{1,27} = 11.66^{**}$	$F_{1,27} = 1.53$ ns	$F_{1,27} = 0.11$ ns	$F_{1,27} = 0.73$ ns	$F_{1,27} = 2.15$ ns
young 'Robust' barley	Head*assay	$F_{1,27} = 9.06^{**}$	$F_{1,27} = 4.23^{*}$	$F_{1,27} = 0.41$ ns	$F_{1,27} = 1.40$ ns	$F_{1,27} = 4.21^{*}$
	Block	$F_{5,27} = 1.60$ ns	$F_{5,27} = 0.72$ ns	$F_{5,27} = 1.72$ ns	$F_{5,27} = 0.97$ ns	$F_{5,27} = 1.35$ ns

[1]F value is significant at *P<0.05, **P<0.01, ***P<0.001; or ns, not significant, P>0.05.

treatment: $F_{1,14} = 0.001$, $P = 0.97$; block: $F_{4,14} = 4.95$, $P<0.05$). In the second test, egg counts on the four head types differed (Welch ANOVA, treatment: $F_{3,56} = 13.81$, $P<0.001$). More eggs were oviposited on young 'Roblin' (mean ± SE = 63.40 ± 11.60) than on old 'Roblin' (20.60 ± 6.21), young 'Key' (14.67 ± 5.63), or young barley (1.20 ± 0.50). In the third test, more eggs were oviposited on young 'Roblin' when males were present in cages with females (90.57 ± 10.96) than when females were without males (50.07 ± 4.83) (ANOVA, male effect: $F_{1,21} = 19.09$, $P<0.001$; block: $F_{5,21} = 8.17$, $P<0.001$). In the fourth test, both time of night and head type had significant effects (ANOVA on log-transformed data: time of night: $F_{1,17} = 35.04$, $P<0.001$; head type: $F_{1,17} = 33.91$, $P<0.001$; time*head interaction: $F_{1,17} = 0.00$, $P = 0.99$; block: $F_{3,17} = 0.70$, $P = 0.56$). Regardless of head type, egg counts were greater before midnight (young 'Roblin', 99.33 ± 25.55; old 'Roblin', 19.83 ± 4.43) than after midnight (young 'Roblin', 22.50 ± 8.51; old 'Roblin', 4.33 ± 0.88).

Behavioral observations

Typically after landing, the female extended the terminal abdominal segments, with the ovipositor visible and in contact with surfaces of the seed head (probing). During probing, the female either walked up and down the seed head or remained in one location. When the female remained in one location, the ovipositor sometimes was visible and contacting exposed head surfaces. At other times, the ovipositor was inserted behind the modified leaves of the seed head. Presumably oviposition occurred at this time. During probing, antennation was observed but could not be scored accurately because of its short duration. Two other behaviors that occurred on the head, walking and sitting, differed from probing in that the tip of the ovipositor was removed from the plant surface and frequently was not visible because the terminal abdominal segments were telescoped.

A preliminary behavioral test compared activity levels of females held singly in cages vs. females held in groups of four. Proportions observed on heads were 4–5 times greater for groups of females regardless of head type (ANOVA, density effect: $F_{1,15} = 28.98$, $P<0.001$; head effect: $F_{1,15} = 3.56$, $P = 0.08$; density*head interaction: $F_{1,15} = 2.16$, $P = 0.16$; block effect: $F_{5,15} = 0.99$, $P = 0.45$).

In the first test comparing young vs. old 'Roblin' wheat (Table 1, Figure 1), head type had a significant effect on percentage on head, percentage probing, percentage sitting, and number of eggs. Assay type, i.e., choice vs. no-choice, had no effect and there was no head*assay interaction. Block effects were significant for four out of five measures. During the 2.5-h observation period, female responses to the two head types were consistent for percentage on heads (Figure 2A) but more variable for percentage probing (Figure 3A).

In the second test comparing young 'Roblin' wheat vs. young 'Key' wheat (Table 1, Figure 4), head type had a significant effect on percentage on head, percentage probing, percentage sitting, percentage walking, and number of eggs. Assay and block effects were not significant and there was no head*assay interaction. During the 2.5-h

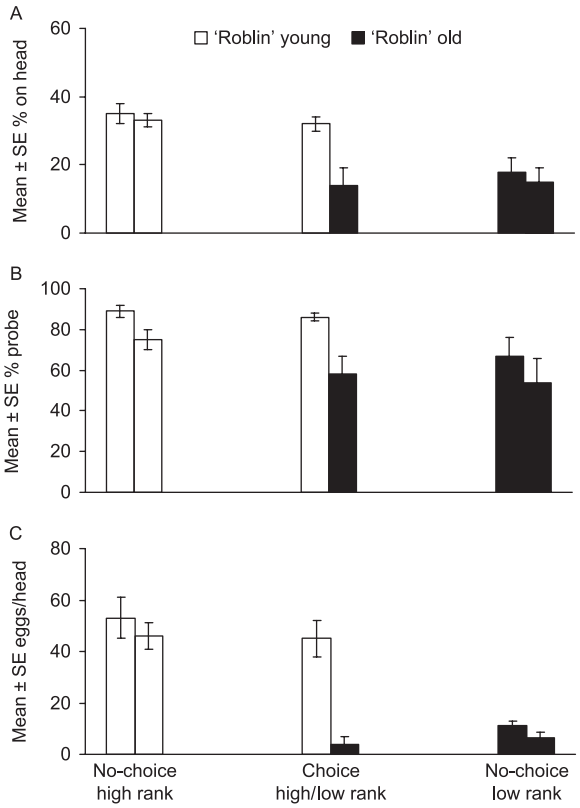

Figure 1 Choice and no-choice assays comparing behaviour of orange blossom wheat midge on young 'Roblin' wheat vs. old 'Roblin' wheat: (A) percentage on head, (B) percentage probing, and (C) eggs per head.

observation period, female responses to the two head types were consistent for both percentage on heads (Figure 2B) and percentage probing (Figure 3B).

In the third test comparing young 'Roblin' wheat vs. young 'Robust' barley (Table 1, Figure 5), head type had a significant effect on percentage on head, percentage probing, percentage sitting, percentage walking, and number of eggs. For percentage on head, the significant assay effect and the assay*head interaction (Table 1) were related to reduced percentage of females on 'Roblin' in the choice test relative to the no-choice 'Roblin' test (Figure 5A), an effect not seen for barley. For percentage probing, the head*assay effect (Table 1) was related to greater percentage probing on barley in the choice test relative to the no-choice test (Figure 5B). This effect was not seen for 'Roblin' wheat. For eggs, the head*assay effect (Table 1) was similar to that seen for percentage probing, i.e., greater eggs on barley in the choice test relative to the no-choice test (Figure 5C). Block effects were not significant (Table 1). During the 2.5-h observation period, female responses to the two head types were consistent for percentage on heads (Figure 2C), but for percentage probing, more probing was observed during the second half of the observation period (Figure 3C).

Discussion

The first aim of our study was to develop methods for observing the small, pale-colored, nocturnally active wheat midge female. The size and color of females made it necessary to create small arenas (4 cm in diameter) for close observation. Excised grass heads were used because they were easier to deploy in small arenas. As had been shown in previous tests, running for up to 3 days (Lamb et al., 2000, 2003; Smith & Lamb, 2001), wheat midge females oviposit similar numbers of eggs on excised vs.

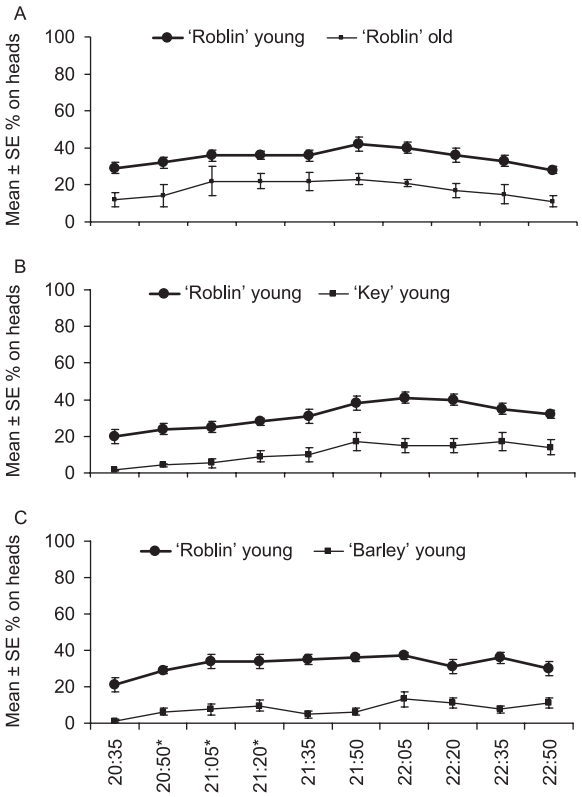

Figure 2 Over the 2.5-h observation period for orange blossom wheat midge, temporal patterns of percentage on head for tests of (A) young vs. old 'Roblin' wheat, (B) young 'Roblin' wheat vs. young 'Key' wheat, and (C) young 'Roblin' wheat vs. young 'Robust' barley. For each time, data from three sample intervals were combined, i.e., 20:35 represents data from observations at 20:30, 20:35, and 20:40 hours. The three times with an asterisk indicate when sunset occurred, i.e., between 20:50 and 21:20 hours.

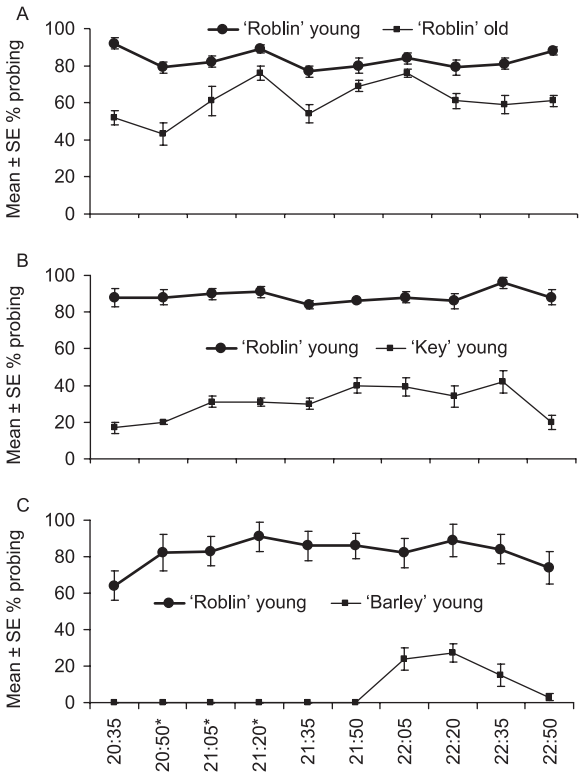

Figure 3 Over the 2.5-h observation period, for orange blossom wheat midge, temporal patterns of percentage probing for tests of (A) young vs. old 'Roblin' wheat, (B) young 'Roblin' wheat vs. young 'Key' wheat, and (C) young 'Roblin' wheat vs. young 'Robust' barley. See Figure 2 for an explanation of time intervals.

non-excised seed heads. The timing of observations, i.e., 20:30–23:00 hours, was based on results of Pivnick & Labbé (1993) and our test (see Results) showing that a significant proportion of eggs are oviposited in the hours before midnight. Although adult wheat midges are known for their nocturnal activity patterns (Pivnick & Labbé, 1993), females visited seed heads during the hour that preceded sunset and, after sunset, did not exhibit new behaviors or show dramatic increases in activity (Figures 2 and 3).

Before conducting the tests, we had planned instantaneous sampling of multiple, individually caged females. This idea was abandoned after discovering that individually caged females show only low levels of activity, typically remaining on the walls of the cage rather than flying and landing on the seed head. When a group of four females was compared to four individually caged females, numbers of females observed on heads increased significantly for both high-ranked and low-ranked heads. Why do groups of wheat midge females show higher activity levels than females held singly? For individual wheat midge females, it seems that the combination of exogenous stimuli from the seed head with endogenous stimuli from the female (Miller & Strickler, 1984) triggers only occasional movement. Having four females in a cage creates opportunities for the occasional movement of one female to trigger movement of other females, with cascading effects. Wheat midge males may have a similar effect because, when caged with males, females oviposited more eggs. Additionally, the presence of a female on a seed head may attract other females. In wheat fields, two or more females on a seed head has been noted even when low female densities make this occurrence unlikely (Pivnick & Labbé, 1992; Smith & Lamb, 2001). Attraction of females to already-occupied seed heads could be mediated by visual stimuli or chemical stimuli, e.g., the release of the female-produced sex pheromone during probing of head surfaces (Gries et al., 2000).

Given low light conditions, the small size of females, and the presence of multiple females on a single head, we

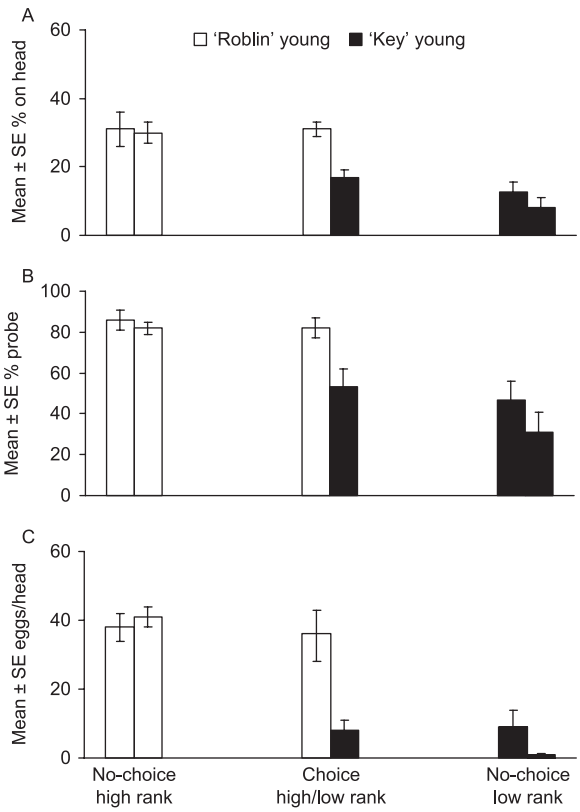

Figure 4 Choice and no-choice assays comparing behavior of orange blossom wheat midge on young 'Roblin' wheat to young 'Key' wheat: (A) percentage on head, (B) percentage probing, and (C) eggs per head.

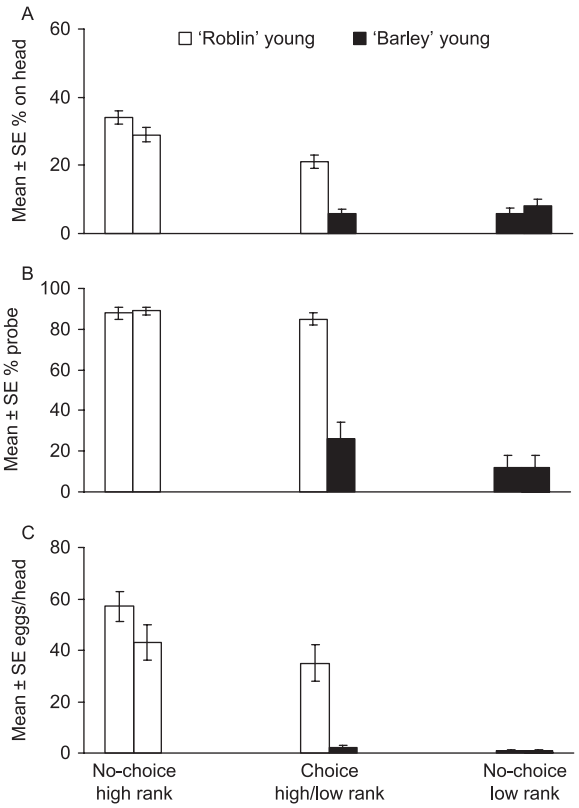

Figure 5 Choice and no-choice assays comparing behavior of orange blossom wheat midge on young 'Roblin' wheat to young 'Robust' barley: (A) percentage on head, (B) percentage probing, and (C) eggs per head.

identified several behaviors that could be scored reliably. These on-plant behaviors are similar to those described for other cecidomyiids, e.g., brassica pod midge, sorghum midge, and Hessian fly (Åhman, 1985; Waquil et al., 1986; Harris & Rose, 1989). Probing is the most obvious examining behavior and occurs when the female extends the terminal abdominal segments (which are normally telescoped into the abdomen) and then bends the abdomen, bringing the tip of the ovipositor down to contact the plant surface. The tip of the ovipositor may be visible while touching the surfaces of the modified leaves that enclose the developing wheat seed or concealed while inserted between the modified leaves. During probing, taste and tactile sensilla on cuticular surfaces surrounding the oviduct (Hallberg & Åhman, 1987) are presumably brought in contact with fine surface features, e.g., ridges and hairs (Kanno & Harris, 2000), and chemicals in epicuticular waxes (Harris & Rose, 1990; Foster & Harris, 1992; Morris et al., 2000). Two other behaviors that were scored, sitting and walking on the head, did not involve examination of plant surfaces with the ovipositor. Antennation and oviposition also occurred but could not be scored reliably.

In each of three tests (Table 1, Figure 1), we compared wheat midge behavior on a pair of high- and low-ranked seed heads in both choice and no-choice settings. Thus, within each replicate of each test, there were three cages, each containing two seed heads and four 2-day-old (presumably mated) females. Seed heads were either in the pre-anthesis stage (young) or in the post-anthesis stage (old). In the first no-choice cage, there were two heads of the high-ranked young 'Roblin' wheat. In the second no-choice cage, there were two heads of one of the three low-ranked seed heads, old 'Roblin' wheat, young 'Key' wheat, or young 'Robust' barley. In the choice cage, there again were two heads, the high-ranked young 'Roblin' wheat and one of the three low-ranked seed heads. Using both choice and no-choice settings is useful for determining whether the high-ranked type stimulates activity on the low-ranked type or whether the low-ranked type inhibits activity on the high-ranked type (Saxena & Basit, 1982). No-choice tests are useful for determining whether female behavior toward low-ranked hosts changes with increasing periods of deprivation of high-ranked hosts (Papaj & Rausher, 1983; Roitberg & Prokopy, 1983; Mangel, 1987).

Egg counts from a choice test had shown the following ranking of seed heads: young 'Roblin' wheat (63 eggs) > old 'Roblin' wheat (21 eggs), young 'Key' wheat (15 eggs), and young barley (one egg). Our behavioral observations showed that this ranking relies, at least in part, on information obtained after the female lands on the seed head. The behavioral measure that showed this most clearly was percentage probing. This was expressed as a proportion of the females observed on the head and, in the three tests, showed progressively greater differences between high- and low-ranked heads (Table 1, Figure 3). The proportion of females observed sitting or walking showed the opposite effect (Table 1), with higher proportions of females engaging in these behaviors on the three low-ranked head types. Typically, females that allot less time to examining and more time to sitting oviposit fewer eggs (Harris & Miller, 1991). We conclude that, after landing on the head, the wheat midge female detects a feature (or features) that determines what happens before she flies away, i.e., whether she continues to examine the head and oviposits many eggs (up to 30 eggs per visit; GASM Ganehiarachchi, unpubl.), or lapses into sitting or walking and either does not oviposit or oviposits only one or two eggs.

Did pre-landing behaviors also contribute to the ranking of seed heads? The measure percentage on head could be an indicator of frequency of landing, especially if females visited heads for only a short time. However, wheat midge visits are typically quite long, e.g., averaging 20, 11, and

5 min on young 'Roblin', old 'Roblin', and young 'Key', respectively (GASM Ganehiarachchi, unpubl.). Because of these long visits, differences in percentage on head for high- vs. low-ranked seed heads could have resulted from differences in visit duration rather than differences in landing frequency. For example, during a 20-min long visit to young 'Roblin', the female would have been scored on the head during 3–4 sample intervals (out of a total of 31 sample intervals). During a 5-min visit to young 'Key', the female would have been scored on the head only once. Tests scoring orientation and landing are needed to determine if wheat midge females distinguish between head types before landing.

The use of simultaneous choice and no-choice tests revealed interesting effects of the low-ranked young barley on the high-ranked young 'Roblin' wheat and vice versa (Table 1). The important comparison here is how females responded to a single head type in the choice test vs. the no-choice test. Overall, the presence of a barley head in the choice test cage reduced the proportion of females (percentage on head) observed on the young 'Roblin' wheat head (Figure 5A). Barley's interference with female responses to young wheat could have occurred either when females were in the cage but not on heads (e.g., by suppressing female flight and/or landing) or when females were on the wheat head (e.g., by inhibiting probing). On the other hand, once the female was on the barley head, the presence of a wheat head in the choice test cage increased probing (Figure 5B) and oviposition (Figure 5C) on barley but did not influence sitting or walking (Table 1). It seems likely that volatile chemicals from the young wheat head (Birkett et al., 2004) stimulate females on barley heads to prolong their examination and eventually oviposit more eggs. Interactions between low- and high-ranked plants have been reported for a number of insect herbivores (Khan et al., 2001) and can play a role in pest management via the push–pull or stimulo–deterrent diversionary cropping strategy (Pickett et al., 1986; Miller & Cowles, 1990).

Our results have created new opportunities for studying the host-finding and selection behavior of the wheat midge female. First, by discovering that females can be observed in small arenas before sunset and are far more active when held in groups, we have paved the way for focal animal sampling. This more detailed analysis of wheat midge behavior will, in turn, further define the plant traits that influence host selection. Second, our results, and the results of other researchers (Lamb et al., 2001; Smith & Lamb, 2001; Birkett et al., 2004), point to a role for plant chemistry in host finding and selection. Based on our results, it seems likely that we will find both stimulatory chemicals produced by high-ranked seed heads and inhibitory chemicals produced by low-ranked seed heads. Moreover, because females examining seed heads appear to be influenced by chemicals directly from the seed head, as well as volatile chemicals from neighbouring seed heads, it will be advisable to use both choice and no-choice settings to study the main and interacting effects of these chemicals.

Acknowledgements

Many people contributed to this manuscript. We are grateful to Robert Lamb, Marjorie Smith, and Ian Wise (Agriculture and Agri-food Canada) for their advice on experiments and for providing 'Key' seeds; Kirk Anderson and Karin Anderson [Department of Entomology, North Dakota State University (NDSU)] for collecting wheat midge larvae from North Dakota wheat fields; Paul Ode (Entomology, NDSU) for advice on statistical methods; and Inger Åhman and an anonymous reviewer for critical reviews of an earlier draft of the manuscript. Wheat midge voucher specimens have been deposited in the NDSU Insect Reference Collection (Fargo, ND, USA). This research was supported by the National Research Initiative of the US Department of Agriculture Cooperative State Research, Education and Extension Service, grant number 2002-35302-12477 'Mechanisms of Host-Plant Selection in the Wheat Midge'.

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