

Behaviour of *Sitodiplosis mosellana* (Diptera: Cecidomyiidae) on spring wheat spikes with and without oviposition deterrence

A.H. Gharalari, M.A.H. Smith, S.L. Fox, R.J. Lamb

Abstract—Wheat, *Triticum* L. (Poaceae), varieties with deterrence to oviposition by the wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), can be useful in reducing seed damage. The behaviour of ovipositing females on spring wheat, *T. aestivum* L., with and without oviposition deterrence was investigated to account for observed differences in oviposition on deterrent and nondeterring hosts. On deterrent wheat, 34% of females landing oviposited compared with 100% of females landing on nondeterring wheat. The sequence of female behaviours just prior to egg-laying on deterrent spikes was similar to that on nondeterring spikes. The length of time required to lay an egg and mean egg-batch size were similar on deterrent and nondeterring wheat, but females spent nearly twice as long on the latter. After landing on deterrent wheat, females took longer to begin ovipositing and longer to leave after the last oviposition event than did females on nondeterring wheat, which further reduced the time available for oviposition on deterrent compared with nondeterring wheat. As a result of these behavioural differences, deterrence reduced oviposition by more than 60%.

Résumé—Les variétés de blé, *Triticum* L. (Poaceae), qui possèdent des mécanismes de dissuasion de la ponte par la cécidomyie du blé, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), peuvent être utiles pour réduire le dommage aux graines. Nous avons examiné le comportement de ponte de femelles sur du blé de printemps, *T. aestivum* L., avec ou sans mécanismes de dissuasion, afin d'expliquer les différences de ponte observées sur les hôtes qui possèdent ou non ces mécanismes de dissuasion. Trente-quatre pour cent des femelles qui se posent sur le blé avec mécanismes de dissuasion pondent, alors que 100 % de celles qui arrivent sur le blé sans mécanismes de dissuasion déposent des œufs. Les séquences des comportements des femelles juste avant et durant la ponte sont semblables sur les épis avec et sans mécanismes de dissuasion. Le temps nécessaire pour la ponte d'un œuf et la taille moyenne des masses d'œufs sont semblables, mais les femelles passent presque deux fois plus de temps sur le blé sans mécanismes de dissuasion que sur le blé qui en possède. Après s'être posées sur du blé avec mécanismes de dissuasion, les femelles mettent plus de temps avant de pondre et y demeurent plus longtemps après la dernière ponte que les femelles sur le blé sans mécanismes de dissuasion, ce qui réduit encore plus le temps disponible pour la ponte sur le blé avec mécanismes de dissuasion par comparaison au blé sans mécanismes de dissuasion. À cause de ces différences comportementales, la dissuasion réduit la ponte de plus de 60 %.

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Introduction

The wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), is a key pest

of wheat, *Triticum* L. (Poaceae), in the northern Great Plains of North America (Lamb *et al.* 1999; Olfert *et al.* 2009). Larval feeding results in kernel shrivelling, which reduces quality and

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quantity of yield (Lamb *et al.* 2000*b*). Oviposition deterrence to female wheat midges has been confirmed in varieties of durum wheat, *T. durum* Desf. (Poaceae) (Lamb *et al.* 2001), and common wheat, *T. aestivum* L. (Lamb *et al.* 2002; Ganehiarachchi and Harris 2007, 2009; Fox *et al.* 2009), and promises to be useful for reducing wheat midge damage. However, this resistance mechanism is controlled by more than one gene, with complementary interaction among genes (Gharalari *et al.* 2009*a*), so incorporating it into breeding programs will be a challenge. Furthermore, the mechanism of oviposition deterrence is not understood, so any increase in our understanding of oviposition deterrence should assist in combining this resistance with antibiotic resistance based on the *Sml* gene (McKenzie *et al.* 2002), which is now being deployed, or in preserving the resistance status of *Sml* using refuges (Smith *et al.* 2004).

Female *S. mosellana* oviposit on wheat spikes between emergence from the flag leaf and flowering (Elliott and Mann 1996). They are active at dusk, preferring warm, calm, humid evenings (Reeher 1945; Pivnick and Labbé 1993). Eggs are laid singly or in small groups in an aggregated distribution among wheat spikes (Smith and Lamb 2001). Observation of wheat midge oviposition behaviour on susceptible and deterrent host-plant spikes has revealed that the length of time a female spends on a spike and her activity level are related to host-plant suitability (Ganehiarachchi and Harris 2007, 2009). Six behaviours are defined for wheat midges as they oviposit on wheat spikes: arriving, probing, inserting the ovipositor, walking, sitting, and departing (Ganehiarachchi and Harris 2007, 2009). All behaviours occur on both deterrent and nondeterrent wheat spikes, although the transitions from one to the next vary with host acceptability: females probe less frequently and insert their ovipositors into spikelets much less frequently on deterrent hosts, and spend less time on deterrent hosts, than on preferred ones (Ganehiarachchi and Harris 2009). Differences in oviposition behaviour on preferred and less-preferred host plants or nonhosts have also been reported for various midges (Diptera: Cecidomyiidae): sorghum midge, *Contarinia sorghicola* Coquillett (Waquil *et al.* 1986), Hessian fly, *Mayetiola destructor*

(Say) (Harris and Rose 1989), and brassica pod midge, *Dasineura brassicae* (Winnertz) (Åhman 1985).

This study describes the behaviour of ovipositing wheat midges on spring wheat (*T. aestivum*) with and without oviposition deterrence, extending the research of Ganehiarachchi and Harris (2007, 2009). The objective was to determine how the different oviposition rates result from differences in behaviour on the two hosts. We focused on the sequence of behaviours leading up to oviposition, the timing of oviposition, and the intervals between oviposition events on the two types of host. Understanding the behaviours that result in oviposition deterrence should identify the plant traits that are cues and improve the breeding of a resistant crop.

Materials and methods

Insects and plants

Wheat midges were obtained from laboratory cultures maintained on 'Roblin', a preferred and susceptible spring wheat (Lamb *et al.* 2000*a*). Laboratory cultures were supplemented annually with field-collected larvae from susceptible wheat. Adults emerged from puparia in containers of moist soil that had been maintained at 2.5 °C for a minimum of 4 months, and were collected in cages held at 20 °C. Adults used in observations were 24–48 h old, by which time females had been inseminated and were ready to lay eggs (Pivnick and Labbé 1993).

Observations were made in the laboratory on 'Roblin' and on 'Key 10', a spring wheat line with oviposition deterrence to wheat midge (Lamb *et al.* 2002). These wheat genotypes were the ones studied by Ganehiarachchi and Harris (2007, 2009). Wheat plants were grown hydroponically in plant-growth cabinets in artificial soil in individual tubular plastic pots (Ding and Lamb 1999).

Observations of oviposition

Observations of adult female wheat midges were made in a controlled-environment room at 20 ± 3 °C, 45%–70% R.H., and a 20 h per day light period, including dawn and dusk intervals of 2 h each, during which light intensity changed gradually. Either 5 spikes of 'Roblin' or

5 spikes of 'Key 10' that had completely emerged from the flag leaf but had not flowered (Zadoks growth stage 59; Zadoks *et al.* 1974) were excised and inserted into Floral Piks (Smith and Lamb 2001). A schematic diagram of a spike with 22 spikelets was used as a data sheet with the spikelets numbered sequentially from 1 to 22, starting from the bottom spikelet. For each spike, the number of the terminal spikelet was recorded. The Piks containing the spikes were placed in a row, 3 cm from each other, on a Styrofoam block and the spikes were misted to provide the adult wheat midges with water. Observations were made during the evening, with the light level decreasing at a rate consistent with that in nature at the time when oviposition normally occurs. A cage 55 cm tall and 25 cm × 25 cm at the base, made of bamboo sticks with walls of plastic film, was placed over the row of spikes. A container of 20 wheat midges, about equal numbers of females and males, was opened into the cage at the bottom, releasing the midges. They initially flew upward toward the top of the cage and usually landed there. As soon as one of the females landed on a spike, the cage was removed to facilitate observation and a stopwatch was set to 0000 and allowed to run continuously until the female left the spike. On-spike females showed no signs of disturbance when the cage was removed. Only one observation (the on-spike activity of one female) was completed per day.

Preliminary observations on 'Roblin' spikes were conducted to identify behaviours associated with oviposition. For each of 10 separate observations, the spikelets into which a female inserted her ovipositor were recorded by marking the corresponding spikelets on a schematic diagram with the times, in minutes and seconds, when she inserted her ovipositor and then withdrew it. Following each observation, spike dissections under a stereomicroscope showed eggs present in spikelets where a wheat midge had remained still for more than a few seconds while her ovipositor was inserted into the spikelet. This behaviour was termed an oviposition event. No eggs were found in spikelets in which the female or her ovipositor continued to move while the ovipositor was inserted. This behaviour was used subsequently to identify spikelets in which a female was ovipositing, thus

refining the "insert" behaviour of Ganehiarachchi and Harris (2009), which did not distinguish between insertions of the ovipositor that did or did not result in oviposition.

For each observation, the times of beginning and ending each oviposition event were recorded on the corresponding spikelets of the spike diagram. The beginning and ending of an oviposition event were recorded as the time when the female inserted and withdrew her ovipositor, respectively. The observation ended when she flew away from the spike. Following each observation, the spike was dissected under a stereomicroscope by removing and dissecting spikelets on which oviposition events had occurred, starting from the lowest numbered spikelet. All surfaces of each spikelet and the associated rachis section were examined to determine the total number of eggs laid.

Statistical analysis

Data were analyzed using procedures of SAS[®] (SAS Institute Inc. 2002). Comparisons of the activity of females on 'Roblin' and 'Key 10' spikes were made using a two-sided *t* test (PROC TTEST) or, for non-normal data, Wilcoxon's two-sample test (PROC NPAR1WAY) as follows: time from landing on a spike until the beginning of the first oviposition event, time from the end of the last oviposition event until the female left the spike, total number of eggs laid on the spike, and number of eggs laid per oviposition event inferred from the observation of a female on a spikelet and the subsequent number of eggs on that spikelet. For 'Roblin' and 'Key 10', correlations (PROC CORR) between the total time spent on a spike and the following variables were performed: total number of eggs laid, time from landing until the beginning of the first oviposition event, and time from the end of the last oviposition event until the female left the spike. For 'Roblin' and 'Key 10', correlations (PROC CORR) between the total number of eggs laid and the following variables were performed: time from landing until the beginning of the first oviposition event, and time from the end of the last oviposition event until the female left the spike.

A repeated-measures analysis (Wolfinger and Chang 1995) was performed using PROC MIXED because observations were repeated

measurements on a single female over time. Prior to analysis, the time a female spent on a spike was divided into consecutive 5-min intervals, hereinafter called time intervals. Time between oviposition events, time required for laying one egg, and number of eggs laid during each time interval were analyzed in separate models. For all three variables, the fixed effects were time interval, wheat line, and the interaction between wheat line and time interval. The repeated statement in the model was assigned for the time interval. The subject was the observation, as each observation was on one midge. Considering the time interval as a continuous variable, a polynomial model was used, with wheat line, wheat line \times time interval, and linear, quadratic, and cubic terms of time interval as fixed effects. For all three variables, the quadratic and cubic terms were not significant ($0.09 < P < 0.84$), therefore a reduced model was used with time interval as a linear effect.

In all tests, differences among treatments were considered significant at $P < 0.05$; values are given as the mean \pm SE.

Results

Description of oviposition behaviour

Observations were made until a total of 27 females were observed to land on 'Roblin' spikes and 65 on 'Key 10' spikes, with each observation made on a single female on one spike in an evening. Oviposition occurred on all 27 'Roblin' spikes but on only 22 'Key 10' spikes. On the other 43 'Key 10' spikes, females did not walk about on the spikes, extend the ovipositor, or probe, and flew away after a median time interval of 1 min 50 s (range: 20s – 14 min 14 s). The behaviour of females was similar on spikes of 'Roblin' and 'Key 10' when they were approaching a spike, and just prior to and during oviposition.

Females approached a wheat spike either by flying directly toward it and landing on the spike or its peduncle, or by circling or zigzagging around the peduncle and landing on the spike or flag leaf. After landing, a female sometimes moved her head toward water droplets on the surface and appeared to drink. In this situation she did not antennate and the

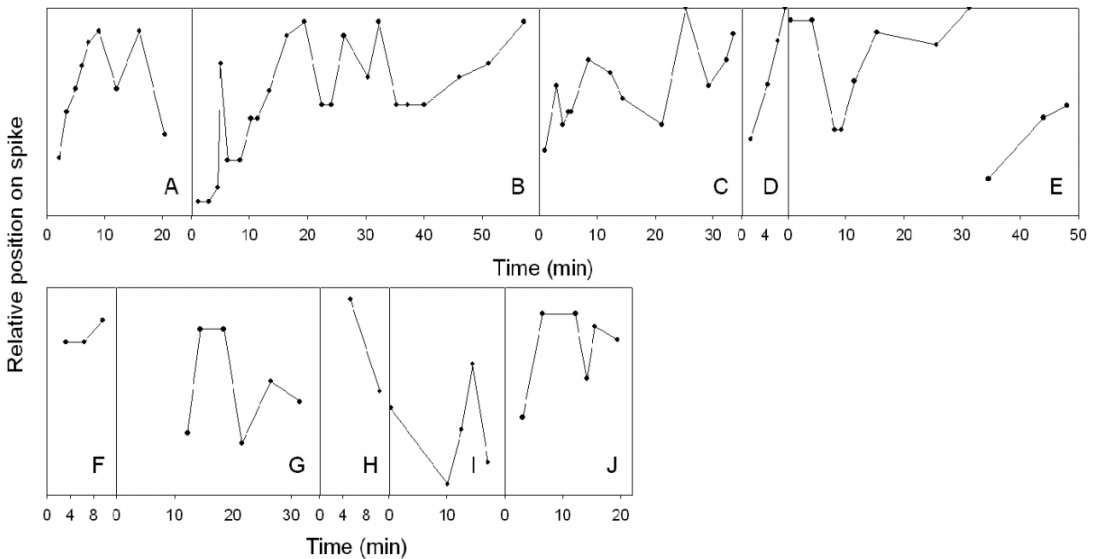
ovipositor was not extended. After moving away from water droplets, she either exhibited oviposition behaviour, or rested on the plant and eventually flew away.

After landing on a spike, a female was motionless, not visibly moving any body parts for a few seconds. Gradually she extended her ovipositor, lowered its tip toward the surface of the spike, and moved it from side to side while tapping the tip on the surface. At the same time, she started antennating at a rate of about 2 times per second. While antennating and moving her ovipositor, she kept her body close to the surface of the spike. When she walked on the spike, she tapped on the surface with her forelegs.

Female wheat midges laid eggs on the rachis, inside the glumes, and inside the florets of spikelets. Before laying eggs inside glumes or florets, a female inserted the tip of her ovipositor between the spikelet structures and moved the tip of her ovipositor from side to side on the inside surfaces. She inserted her ovipositor farther down between the spikelet structures while her legs were on the spikelet surface, moving her body and wings from side to side. Once her ovipositor was inserted, she remained still and did not antennate. After oviposition, she moved her body from side to side and retracted her ovipositor from the spikelet. While she laid eggs on the rachis, her legs were on the lateral parts of the spikelet. She bent her ovipositor about 90° toward the rachis, moved her body from side to side, then remained still and did not antennate. After oviposition, she moved her body from side to side and retracted her ovipositor.

The sequences of spikelets on which wheat midges oviposited as they moved up and down the spike are shown graphically for a representative sample of observations on 'Roblin' (Figs. 1A–1E) and 'Key 10' (Figs. 1F–1J). On 'Roblin', most of the oviposition events were observed when females were walking upward on the spike (Figs. 1A, 1B, 1D), whereas on 'Key 10', females more often changed direction after ovipositing (Figs. 1G, 1I, 1J). When, after ovipositing, a female moved to a lower spikelet to oviposit again, she almost always moved back up to a higher spikelet for the next oviposition. In 6% ($n = 308$) of oviposition

Fig. 1. Representative sample of movement patterns of adult female *Sitodiplosis mosellana* observed in the laboratory on spikes of ‘Roblin’ (A–E) and ‘Key 10’ (F–J) wheat selected from a total of 27 observations on ‘Roblin’ and 22 observations on ‘Key 10’. Each graph shows one observation, which was the movement of one female from landing on until departure from a wheat spike. Each dot on a graph represents an oviposition event. The time axes for all graphs are at the same scale. The y-axes are relative scales extending from the bottom spikelet to the terminal spikelet; the actual number of spikelets in a spike varied from 15 to 22. The line joining the dots represents females walking between spikelets, whereas the absence of a line (E) represents a female flying and then reinitiating oviposition on the same spike.



events on ‘Roblin’ and 3% ($n = 107$) on ‘Key 10’, a female continued down the spike to oviposit again (Fig. 1D), and only once (on ‘Roblin’) did she oviposit on several lower spikelets in succession. When she reached the terminal spikelet, she waved her front legs a few times in the air, then turned and walked toward the lower parts of the spike. In a few instances, a female at or near the top of a spike flew downward in a spiral a few centimetres from the spike, landed on a spikelet lower down, and oviposited again (Fig. 1E). When she was ovipositing, her head was usually oriented upward; rarely, her head was oriented downward.

After the last oviposition event, a female remained in position on the spike, retracted her ovipositor, and did not antennate. After a while she flew away and the observation was ended. Activity of an individual female was observed on only one spike, so if she came back and landed on another spike, the observation was discontinued.

Quantitative aspects of oviposition behaviour on ‘Roblin’ and ‘Key 10’ wheat spikes

Female wheat midges on ‘Roblin’ stayed 1.75 times longer ($t = -3.83$, $P = 0.0004$, $df = 44.3$) and laid 2.5 times more eggs ($t = -5.73$, $P < 0.0001$, $df = 40.9$) than on ‘Key 10’ (Table 1). There were 3–26 (range) oviposition events per observation on ‘Roblin’ compared with 1–10 on ‘Key 10’, with similar numbers of eggs laid during each oviposition event (Table 1) ($t = -0.43$, $P = 0.6703$, $df = 47$). The within-spike position of the first oviposition event differed between ‘Roblin’ (15, 8, and 4 in the lower, middle, and upper third of the spike, respectively) and ‘Key 10’ (7, 4, and 11 in the lower, middle, and upper third of the spike, respectively) ($\chi^2 = 7.07$, $P = 0.0291$, $df = 2$).

The mean time from landing on a spike until the first oviposition event was shorter on ‘Roblin’ than on ‘Key 10’ ($t = -5.73$, $P < 0.0001$, $df = 40.9$). The mean time from the end of the last oviposition event until

Table 1. On-spike activities (mean \pm SE) of female *Sitodiplosis mosellana* on spikes of nondeterrent ‘Roblin’ spring wheat and deterrent ‘Key 10’ spring wheat.

	‘Roblin’	‘Key 10’
No. of observations	27	22*
Total time spent on spike (min)	34.5 \pm 3.2	19.6 \pm 2.2
Time from landing to beginning of first oviposition event (min)	1.5 \pm 0.2	4.1 \pm 0.6
Time from end of last oviposition event to leaving spike (min)	1.9 \pm 0.5	2.7 \pm 0.4
Total no. of eggs laid	25 \pm 2	10 \pm 1
No. of eggs laid per oviposition event	2.2 \pm 0.9	2.1 \pm 0.1

Note: An observation of a female lasted from the time of landing on a wheat spike until departure.

*Only spikes on which eggs were laid were included in the analysis (22 of a total of 65 observations).

departure from the spike was shorter on ‘Roblin’ than on ‘Key 10’ (Wilcoxon’s test, $Z = 2.57$, $P = 0.0101$) (Table 1).

The total number of eggs laid increased with the total length of time spent on a spike for both ‘Key 10’ ($r_p = 0.76$, $P < 0.0001$) and ‘Roblin’ ($r_p = 0.65$, $P = 0.0002$). The time from landing on the spike until the first oviposition event was independent of the total number of eggs laid on either ‘Key 10’ ($r_p = 0.02$, $P = 0.9164$) or ‘Roblin’ ($r_p = 0.20$, $P = 0.3209$) and, similarly, the time from the last oviposition event until departure was independent of the total number of eggs laid on either ‘Key 10’ ($r_p = -0.02$, $P = 0.9157$) or ‘Roblin’ ($r_p = -0.19$, $P = 0.3334$). The time spent on a spike increased with the time from landing on a spike until the first oviposition event for ‘Key 10’ ($r_p = 0.49$, $P = 0.0193$) but not for ‘Roblin’ ($r_p = 0.02$, $P = 0.8874$). No relationship was detected between the total length of time spent on a spike and the time from the end of the last oviposition until the female departed, for either ‘Key 10’ ($r_p = 0.13$, $P = 0.5479$) or ‘Roblin’ ($r_s = 0.07$, $P = 0.7018$).

The number of eggs laid during 5-min intervals and the time elapsed between oviposition events changed over consecutive time intervals (Table 2). Only the number of eggs laid during time intervals differed between ‘Roblin’ and ‘Key 10’ (Table 2). On both ‘Roblin’ and ‘Key 10’, the time elapsed between oviposition events increased over time. Although the differences between ‘Roblin’ and ‘Key 10’ were not significant, there was a trend, between 5 and 35 min after the observation began, for the time

between oviposition events to be longer for ‘Key 10’ than for ‘Roblin’ (Fig. 2A). The mean number of eggs laid during a time interval decreased over time and was higher on ‘Roblin’ than on ‘Key 10’ (Fig. 2B). The length of time required to lay one egg (duration of oviposition event divided by number of eggs) did not differ between ‘Roblin’ and ‘Key 10’, and did not change over time (Table 2, Fig. 2C).

Discussion

Previous observations of female wheat midges on wheat spikes revealed patterns in a set of behaviours associated with the search for oviposition sites and with oviposition activity (Ganehiarachchi and Harris 2007, 2009). Our study focused more specifically on the sequence, timing, and duration of oviposition events; however, some of the previously observed behaviours were also seen—specifically, antennating, probing surfaces with the extended ovipositor, and inserting the ovipositor between spikelet surfaces. Ganehiarachchi and Harris (2007, 2009) did not report the duration of ovipositor insertion, which they assumed to be equivalent to oviposition. We distinguished between two behaviours: insertion without oviposition and insertion with a pause. The latter behaviour only resulted in the deposition of eggs if it included a multisecond pause when the female and her ovipositor were still, during which we concluded that eggs were deposited. If the ovipositor was withdrawn without a pause, there was no oviposition, suggesting

Table 2. Repeated-measures mixed-model analysis of three aspects of oviposition on wheat by *Sitodiplosis mosellana*.

Model effect	Time between oviposition events		No. of eggs laid during consecutive 5-min time intervals		Time required to lay one egg	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Time interval*	$F_{1,318} = 36.40$	<0.0001	$F_{1,196} = 7.82$	0.0057	$F_{1,364} = 0.51$	0.4775
Wheat line [†]	$F_{1,44} = 1.10$	0.2990	$F_{1,47} = 5.07$	0.0291	$F_{1,47} = 0.24$	0.6293
Time interval × wheat line	$F_{1,318} = 0.57$	0.4504	$F_{1,196} = 0.36$	0.5471	$F_{1,364} = 1.38$	0.2408

Note: Compound symmetry covariance structure was applied. Time interval was considered to be a continuous variable.

*Consecutive 5-min time intervals.

[†]'Roblin' and 'Key 10'.

that oviposition cues were inadequate, or alternatively, that deterrent cues were detected and the site was avoided. This sequence of probing the surface, inserting the ovipositor, and remaining still for a time while ovipositing has also been described for the brassica pod midge (Åhman 1985) and sorghum midge (Waquil *et al.* 1986).

On both deterrent and nondeterrant wheat, females repeatedly tapped spikelet surfaces with their extended ovipositor while antennating the surfaces, as described previously (Ganehiarachchi and Harris 2009). Female Hessian flies also antennate and probe with the ovipositor tip while searching leaf surfaces for oviposition sites (Harris and Rose 1989). Sensory structures at the tip of the ovipositor of the brassica pod midge have gustatory and tactile functions that likely help a female locate and assess oviposition sites (Hallberg and Åhman 1987). The blackcurrant leaf midge, *Dasineura tetensi* (Rubsaaen) (Diptera: Cecidomyiidae), has mechanoreceptors and chemoreceptors on its antennae that are believed to be involved in host-finding (Crook and Mordue 1999). As is the case for other gall midges, probing plant surfaces probably enables female wheat midges to obtain chemical and tactile cues that indicate whether a suitable oviposition site has been located.

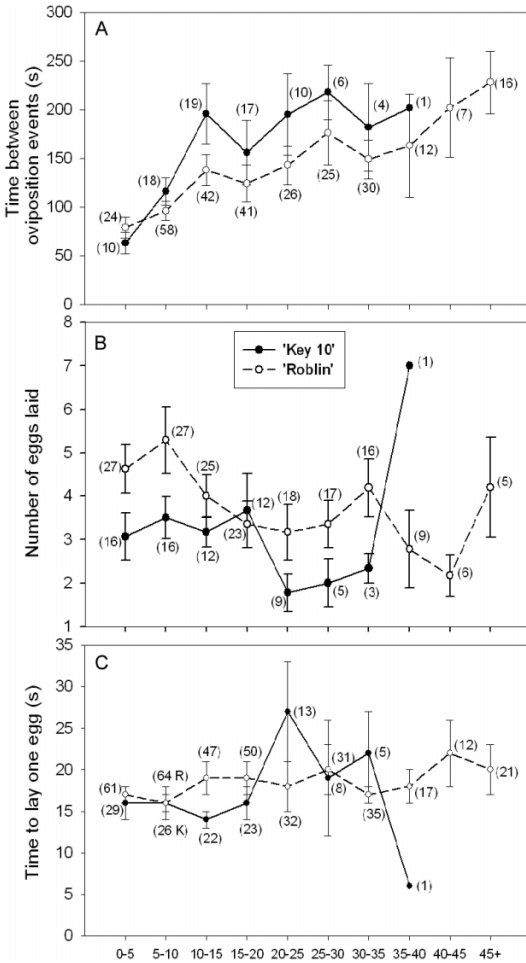
Once a female initiated oviposition, her behaviour was the same on deterrent and nondeterrant hosts, suggesting that egg release and deposition are largely under the control of physiological processes within the reproductive system rather than chemical or tactile cues

received by the female from the plant. Females laid batches of two eggs, on average, per oviposition event, regardless of the wheat type. Lamb *et al.* (2001) report similar-sized egg batches ranging from 1.6 to 2.7 on deterrent and nondeterrant *T. durum* in both field and laboratory. Similarly, the length of time required to lay an egg did not differ between deterrent and nondeterrant wheat and did not change in successive oviposition events. After each oviposition event, the time elapsed before the next oviposition occurred on the same spike became progressively longer, possibly because eggs needed to move farther along the ovarioles before being released.

The most noticeable differences in the oviposition behaviour of wheat midges on 'Key 10' and 'Roblin' observed in this study, and also by Ganehiarachchi and Harris (2009), were in the length of time spent on a spike and the total number of eggs laid while on a spike. Females spent about half as long and laid 57% as many eggs on deterrent 'Key 10' as on nondeterrant 'Roblin'. If the spikes on which no eggs were laid are taken into account, only 20% as many eggs were laid on 'Key 10' as on 'Roblin' because females oviposited on only one third of the deterrent spikes they landed on, whereas they oviposited on all of the nondeterrant spikes they visited. Rejection of deterrent spikes for oviposition by females after landing was also observed by Ganehiarachchi and Harris (2009).

Previous research reported a positive association between number of eggs laid per spike and length of time spent on a spike (Ganehiarachchi and Harris 2009). Our study elucidates

Fig. 2. Time between oviposition events, numbers of eggs laid, and time required for laying one egg during consecutive 5-min time intervals of on-spike activity by adult female *Sitodiplosis mosellana* on 'Roblin' and 'Key 10' wheat spikes. All values are given as the mean \pm SE. Numbers in parentheses show the sample size.



this relationship by demonstrating that the reduction in oviposition on deterrent compared with nondeterrent wheat spikes resulted from the shorter time spent on the spikes. The length of time required to deposit an egg, average egg-batch size, and time between oviposition events on deterrent and nondeterrent wheat were the same, so the number of eggs laid varied largely according to length of time spent on the spikes. Densities of brassica pod midge eggs are higher

on high-quality than on low-quality hosts because of a higher frequency of oviposition of similar-sized egg batches (Åhman 1985). The Hessian fly may show similar behaviour: females stay longer on a preferred host plant and lay more eggs (Harris and Rose 1989). For the wheat midge, a small part of the difference in oviposition on deterrent and nondeterrent wheat resulted from females taking longer after landing to begin ovipositing and longer to leave after the last oviposition event on the deterrent wheat than on the nondeterrent wheat, reducing the time available for oviposition on the deterrent wheat. The delay in the onset of oviposition on deterrent wheat may occur because females are less able to detect the immediate cues that initiate oviposition than is the case on nondeterrent wheat. On the other hand, oviposition may be suppressed by the detection of deterrent volatile compounds or other negative cues. The delay in departing from deterrent wheat may reflect a behavioural conflict between the lack of suitable oviposition cues from the plant, or the detection of repellent cues, and the sensory cues from a female's reproductive system indicating that eggs are still available to be laid.

What cues do female midges receive that influence whether and how long they oviposit on a host plant? Females do frequently land on deterrent wheat, which shows that cues operating at a distance, such as plant volatiles, are not adequate to let females discriminate between deterrent and nondeterrent plants. Once they have landed, females often reject deterrent plants for oviposition before extending their ovipositor and probing as described above, possibly using tarsal sensors, although what plant traits are detected is unknown. Once the ovipositor is extended and plant probing is initiated, some oviposition usually occurs, although the frequency and total number of oviposition events are reduced on deterrent wheat. No morphological structures of wheat spikelets have been found that are clearly associated with deterring wheat midge oviposition (Gharalari *et al.* 2009b). For 'Key 10', which is highly antibiotic (Lamb *et al.* 2000a), the cues that deter oviposition are adaptive for the wheat midge, but that is not always the case. Female wheat midges oviposit

less than expected on some host plants on which their larvae perform well (Lamb *et al.* 2001, 2003), suggesting the oviposition cues are not genetically linked with the antibiotic traits of the plant.

Studies of the chemical and physical characteristics of oviposition sites of some cecidomyiids suggest that stimulant or repellent volatiles, or fine structure and texture of the surface, may be involved. Oviposition is stimulated in the Hessian fly by volatile components of leaf-surface waxes (Foster and Harris 1992) and in the sorghum midge by chemostimuli from fertile sorghum pollen (Sharma *et al.* 1990). Several volatile compounds from pre-flowering wheat spikes, probably in a particular ratio, attract female wheat midges (Birkett *et al.* 2004). The Hessian fly uses visual, chemical, and tactile cues, and the oviposition rate is highest when all these cues are present (Harris and Rose 1990). In the onion fly, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae), visual, structural, and chemical cues act synergistically to stimulate oviposition (Harris and Miller 1982). For the female wheat midge, at least three points in the behavioural sequence leading to oviposition are subject to deterrence: locating and landing on the plant, accepting the plant and initiating probing, and initiating an oviposition event. Now that this behavioural sequence and its contribution to oviposition are better understood, the deterrence mechanisms and their genetic basis should be more amenable to study.

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