

# Seed damage by *Sitodiplosis mosellana* (Diptera: Cecidomyiidae) to spring wheat cultivars with the *Sm1* gene

Ian L. Wise,<sup>1</sup> Stephen L. Fox, Marjorie A.H. Smith

**Abstract**—Six resistant spring wheat cultivars with the *Sm1* gene were assessed for seed damage by the orange wheat blossom midge (*Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae)) in laboratory and field tests. All resistant cultivars deterred larvae from developing on the seed and had significantly less yield losses than susceptible wheat cultivars. Vesper was the first cultivar with *Sm1* that also deterred oviposition by the wheat midge. Seed damage to all resistant cultivars decreased the later plants were exposed to adult midge from the time spikes emerged from the boot until anthesis. Spikes of susceptible wheat cultivars had lower yield losses when exposed two or more days after emergence than spikes exposed at the time of emergence. Seed damage to resistant wheat caused dorsal and lateral distortions of the seed and often altered seed colour and shape. The pedigree of the resistant cultivars had no effect on the extent of seed damage. Shaw wheat had the least amount of seed damage and no third instars on the seed in both field and laboratory tests. All other cultivars had a few small third instars and similar levels of seed damage in laboratory tests, with Fieldstar being the least effective. In at least one mean site-year Shaw had significantly less yield losses than the other resistant cultivars. It is recommended that Shaw be used as the standard for the selection of future spring wheat cultivars with *Sm1*.

## Introduction

The orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) damages spring wheat, *Triticum aestivum* Linnaeus (Poaceae) in wheat-producing regions of western Canada (Smith *et al.* 2014), and in the northern United States of America (Harris *et al.* 2003) and many countries of Europe and Asia (Barnes 1956; Ni and Ding 1994). Damage is highly variable each year (Smith *et al.* 2014), and is characterised by local outbreaks (Olfert *et al.* 1985; Lamb *et al.* 1999).

The larvae of this midge cause economic losses to wheat by completely or partially destroying the seed or by reducing seed grade by the presence of damaged seed in the harvested grain (Lamb *et al.* 2000b). Wheat plants incur damage if females of *S. mosellana* lay eggs on spikes from the time the spikes emerge from the boot until anthesis (Mukerji *et al.* 1988). Females will lay eggs on spikes, at diminishing rates, for up to 10 days after

anthesis, but survival of newly hatched larvae on susceptible wheat begins to decline if eggs are laid during or post-anthesis (Ding and Lamb 1999).

In the 1990s a gene named *Sm1* (McKenzie *et al.* 2002) was discovered that confers antibiosis in wheat to the wheat midge. The gene works by increasing its production of phenolic acids in response to feeding on the seed surface by newly hatched midge larvae (Ding *et al.* 2000). Larvae depart from their feeding site within a few days and die of starvation. The antibiotic effects of resistant wheat on midge larvae are associated with a hypersensitive reaction by the plant at the feeding site on the seed surface (Lamb *et al.* 2000a). Some surface scarification and cell damage at the point of feeding may occur, resulting in small misshapen seed in harvested samples (Lamb *et al.* 2000a). This type of seed injury is used by the authors to identify midge-resistant wheat lines with the *Sm1* gene in grain samples from spring wheat breeding programmes at the Cereal Research Centre (Winnipeg, Manitoba, Canada).

Received 27 October 2014. Accepted 27 October 2014. First published online 24 February 2015.

I.L. Wise,<sup>1</sup> S.L. Fox, M.A.H. Smith, Department of Entomology, 214 Animal Science Building, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

<sup>1</sup>Corresponding author (e-mail: iwise@shaw.ca).

Subject editor: Kevin Floate

doi:10.4039/tce.2014.75

The objective of the study was to measure what effect seed injury to resistant spring wheat by *S. mosellana* has on yield losses to various resistant spring wheat cultivars. Included in this objective is the effect crop stage at the time of oviposition, and subsequent initial feeding by larvae, has on the severity of seed damage to spring wheat cultivars and advanced lines with the *Sm1* resistance gene.

## Materials and methods

### Cultivars

Six Canada western red spring wheat cultivars with the *Sm1* resistance gene, Shaw (Fox *et al.* 2013), Fieldstar (Fox *et al.* 2012), Unity (Fox *et al.* 2009), Goodeve (DePauw *et al.* 2009), Vesper (Thomas *et al.* 2013), and CDC Utmost (unpublished) were included in laboratory and field tests. Two advanced midge-resistant lines, BW314 and BW430, were included in laboratory tests to compare the source of *Sm1* as a potential factor in differences in seed damage to resistant wheat cultivars. These advanced lines and Shaw derived *Sm1* from the winter wheat cultivar Howell (Kolb and Brown 1992). The winter wheat cultivar Clark (Ohm *et al.* 1988) was the source of *Sm1* for Fieldstar, Unity, and Goodeve. Vesper acquired *Sm1* from the winter wheat cultivar Augusta (Everson *et al.* 1986), and *Sm1* in CDC Utmost came from the winter wheat cultivar Seneca (Briggle and Reitz 1963).

The spring wheat cultivars CDC Teal (Hughes and Hucl 1993), Katepwa (Campbell and Czarniecki 1987a), and AC Intrepid (DePauw *et al.* 1999) were grown as susceptible checks in laboratory tests in 2008 and 2009, Roblin (Campbell and Czarniecki 1987b) in 2011, and CDC Teal in 2012. AC Barrie (DePauw *et al.* 1997) was grown as the susceptible check in the field tests. These cultivars were selected because of their known susceptibility to the wheat midge, their extensive past and present commercial use in western Canada, and their spike emergence dates mostly overlap those of the resistant cultivars.

### Laboratory tests

Plants at different stages of spike development were exposed to ovipositing midge. For this purpose, plants were grown individually in plastic tubular pots (Stuewe & Sons Inc., Corvallis,

Oregon, United States of America) with an artificial soil mixture in a growth chamber (Ding and Lamb 1999). The pots were partially submerged in a liquid fertiliser medium (15 g of PlantProd<sup>®</sup> Classic 20:20:20 N-P-K, Master Plant-Prod Inc., Brampton, Ontario, Canada; in 10 L of water). Spikes were labelled with the date when they were at least three-fourths emerged from the boot. Labelling of emerging spikes continued each day for five days after the emergence of the first spike for a possible total of six different dates or spike development stages (DS 0–5) per plant: 0 days: spike three-fourths emerged from boot (Zadoks 57), one day: spike completely emerged from boot with peduncle exposed (Zadoks 58), two days: anthers green and peduncle > 20 mm long (Zadoks 59), three days: anthers yellow and peduncle > 30 mm long (Zadoks 60), four days: initial anthesis (anthers extruded) on some spikelets (Zadoks 61–64), five days: > 50% of spikelets flowering (Zadoks 65–67) (Tottman and Makepeace 1979). Later stages were excluded because of reduced larval establishment on the seed of susceptible wheat (Ding and Lamb 1999), even though females are known to oviposit on spikes after all spikelets have flowered.

Plants with an average of five to six spikes at various spike development stages were transferred to cages in a room with lighting, temperature, and humidity controls (Ding and Lamb 1999) for exposure to the midge. Tillers without emerged spikes or with spikes > 20 cm taller than other spikes on the plant were severed at the crown. Plants were elevated in the cage, if necessary, to minimise differences in spike height among plants. A total of 20–25 plants were added to each cage and 5–10 replicate cages were used in tests in 2008, 2009, 2011, and 2012. At least one susceptible check cultivar, with plants having the same spike emergence times as the resistant plants, where possible, were included in all cages.

A few minutes after plants were placed in cages, newly emerged adult midge ( $1 \pm 0.5$  females per spike) from a laboratory colony were added. After two days, plants were removed from the cages and spikes were either excised at the peduncle or were covered with pollen bags to maintain high humidity near the spike. The excised spikes were kept at 5–10 °C until being examined within 48 hours for eggs with a light microscope. Plants with covered spikes were

moved to a greenhouse with supplemental fluorescent lighting.

The plants in the greenhouse were grown until seeds were mature. Spikes were removed from the plants and the number of third instars (Gagné and Doane 1999) and undamaged (U) and damaged seeds per spike were counted. Midge-damaged seeds were separated by weight as being damaged (> 8 mg) or lost (< 8 mg). The weight separation was determined from the machine harvesting and cleaning of spring wheat plots in Saskatchewan and Manitoba, Canada by the authors in 2006. Damaged (D) seed were retained when spikes were harvested while lost (L) seed were removed.

Yield loss (YL) by the wheat midge for each spike was calculated by:

$$YL(\%) = 100 \times \frac{[(U_{Swt} - D_{Swt}) \div U_{Swt}] \times D + L}{D + L + U}$$

where  $U_{Swt}$  was 1000 seed weight of U seed,  $D_{Swt}$  was 1000 seed weight of D seed, and D, L, and U were the number of each seed per spike.

Egg counts and yield losses were taken from spikes in 2008, 2009, 2011, and 2012, and counts of third instars in all years except 2011. Each spike developmental stage (DS) was replicated 3–10 times per year for each resistant wheat cultivar or line, except DS 5 where only one to seven replicates were used. At each DS, the mean number of spikes per replicate varied from 1.4 to 2.8 per year for each resistant wheat and from 1.8 to 4.3 per year for the susceptible checks. Susceptible plants with spikes at all developmental stages were added to every cage each year, except in 2008 and 2012 where a total of five cages did not include DS 5 spikes.

Data for the three susceptible checks in each cage for 2008 and 2009 were pooled and results for each check cultivar were weighted equally for identical exposure dates. This was also done for each resistant cultivar or advanced line where there was more than one spike of the same developmental stage in a cage. Egg and larval counts and yield losses between wheat cultivars and lines for each exposure date in all years were analysed using procedures of SAS<sup>®</sup> (SAS Institute Inc. 1999). Because all wheat cultivars or lines were not present in all cages, counts of eggs and larvae and yield losses for each wheat were compared with other cultivars or lines in the same cages by the least-squares means test with the

conservative Tukey-Kramer adjustment. Values are reported as the mean  $\pm$  SE with differences between cultivars or lines considered significant at  $P \leq 0.05$ .

For each cultivar and advanced line, the effect of spike developmental stage at time of midge exposure on yield loss data ( $y$ ,  $\log y$ ) was assessed in all years with a linear regression (SAS Institute Inc. 1999). The regression,  $y$  or  $\log y$ , with the highest relationship for most of the resistant wheat cultivars or advanced lines was retained. Where no significant differences were found in the slopes ( $b$ ) and intercepts ( $a$ ) among years by analysis of covariance, the means for 2008 and 2009 and for 2011 and 2012 were pooled for each cultivar.

In laboratory tests of 2012, the seed from five spikes of Vesper, Shaw, CDC Utmost, and the check were examined 8–12 days after exposure. The number of larvae and their stage and location on the spike, and the number of infested and non-infested seed and symptoms of seed damage were recorded.

### Field tests

Resistance of cultivars to the midge was assessed at a minimum of five field sites for at least two years. Six field sites each year were located in Saskatchewan at Saskatoon (52°11'N, 106°32'W), Regina (50°24'N, 104°34'W), Scott (52°21'N, 108°50'W), Indian Head (50°32'N, 103°38'W), Melfort (52°49'N, 104°37'W), and Outlook (51°28'N, 107°03'W) in 2009–2011 as part of the Saskatchewan Variety Performance Group evaluation of the agronomic suitability of crop varieties in various parts of the province.

At each site the resistant cultivars were sown as a varietal blend of 90% resistant and 10% susceptible components, as recommended to conserve wheat midge resistance (Smith *et al.* 2004). Blocks were sown 10–22 May with a double disc press drill and were replicated three times in a randomised complete design. Plots were four or five rows wide and 3.7–5.0 m in length. Granular fertilisers were applied at recommended rates before seeding and foliar herbicides were applied, if necessary, early in the growing season for weed control. No insecticides were applied.

Wheat spikes were collected randomly near crop maturity from each plot, and 10 spikes per plot were examined for seed damage ( $n = 30$

spikes/cultivar). Undamaged (U), damaged (D), and lost (L) seeds were counted on each spike, and U and D seeds were weighed and recorded. Yield losses were computed using the formula as for the laboratory data. Since our objective was to measure the effect of feeding injury to resistant seed, only spikes from the resistant component of the varietal blends were included in the estimation of yield losses to resistant cultivars.

Differences in yield losses by the midge between susceptible and resistant cultivars and within resistant cultivars at the field sites were determined by least-squares means with the Tukey-Kramer adjustment at a significance level of  $P < 0.05$ . Statistical results are included where differences were significant.

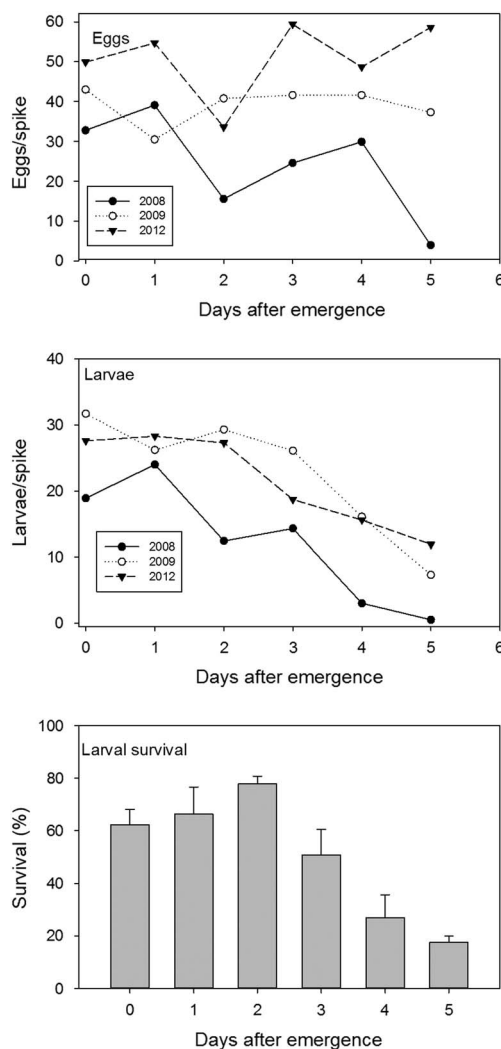
## Results

### Eggs per spike in laboratory tests

In the laboratory tests in 2008, an insufficient number of spikes were examined for eggs to determine differences in egg counts between spike developmental stages within each resistant cultivar or advanced line. A minimum of three replicates were used for the six spike developmental stages of all wheat lines in 2009 and five replicates in 2011 and 2012. No differences were found in the number of eggs laid on each developmental stage (DS) for all resistant wheat lines in 2009, 2011, or 2012. Fewer eggs were laid on spikes of the susceptible checks at DS 5 than on earlier stages in 2008 but no differences were found in 2009 and 2012 (Fig. 1) or in 2011. This enabled egg counts at the different developmental stages for each resistant cultivar or line and for the susceptible cultivars in each cage in all years to be pooled.

An average of 30–50 eggs per spike was laid on the checks in 2008, 2009, and 2012, and over 90 per spike in 2011 (Table 1). All resistant cultivars except Goodeve received fewer eggs than the checks in 2008, but only egg counts for Shaw were significantly less (Table 1). In 2009, BW430 had the fewest eggs but counts, including those for Shaw, were not significantly less than those of the checks (Table 1). Only Vesper had significantly fewer eggs ( $P \leq 0.02$ ) than the check in more than one laboratory test. In 2011 Vesper received over four-fold fewer eggs than Roblin and three-fold fewer than CDC Teal in 2012 (Table 1).

**Fig. 1.** The effect of developmental stage (DS) from initial emergence of spikes to anthesis (DS 0–5) on the number of eggs and larvae per spike and on larval survival (%) of *Sitodiplosis mosellana* in laboratory tests when spikes of the susceptible spring wheat cultivars CDC Teal, Katepwa, and AC Intrepid in 2008 and 2009, and CDC Teal in 2012 were exposed to adults zero to five days after emergence of the spikes.



### Seed damage symptoms to resistant cultivars

A total of 10–14 larvae were on the spikes of Vesper, Shaw, CDC Utmost, and the check when spikes were examined 8–12 days after exposure to adult midge in cages. All larvae on the check were located on the surface of the seed where the seed was damaged and were second instars. Larvae on the resistant cultivars were either dead at the base

**Table 1.** Number of eggs of *Sitodiplosis mosellana* per spike ( $\pm$ SE) on spikes of spring wheat cultivars and advanced lines with *Sm1* when exposed to adults zero to five days after the emergence of the spikes (DS 0–5) in four laboratory tests.

Wheat	2008			2009		
	Cages	Spikes	Eggs $\pm$ SE	Cages	Spikes	Eggs $\pm$ SE
Fieldstar	3	11	14.1 $\pm$ 6.2	7	20	33.0 $\pm$ 13.2
Shaw	4	16	10.1 $\pm$ 4.7*	8	35	35.9 $\pm$ 19.9
Unity	3	7	24.3 $\pm$ 15.4	8	40	22.5 $\pm$ 9.4
Goodeve	5	12	42.2 $\pm$ 7.1	7	34	38.1 $\pm$ 10.7
Check	7	46	31.6 $\pm$ 6.7	10	129	39.2 $\pm$ 8.5
BW314				8	28	35.7 $\pm$ 10.9
BW430				7	43	16.7 $\pm$ 6.3
Wheat	2011			2012		
	Cages	Spikes	Eggs $\pm$ SE	Cages	Spikes	Eggs $\pm$ SE
Vesper	5	39	22.3 $\pm$ 5.4*	7	57	17.0 $\pm$ 2.9*
Shaw	5	21	61.3 $\pm$ 13.4	7	57	41.0 $\pm$ 6.6
Check	5	21	96.6 $\pm$ 26.9	7	52	51.2 $\pm$ 5.0
CDC Utmost				5	43	44.8 $\pm$ 4.4

\**P* (Least-squares means test with Tukey-Kramer adjustment) difference from check (*P* < 0.05).

of the seed or on the glumes, first instars on the seed surface or upper fringe of early exposed spikes (Fig. 2), or were first or second instars on late aborted seed on later exposed spikes.

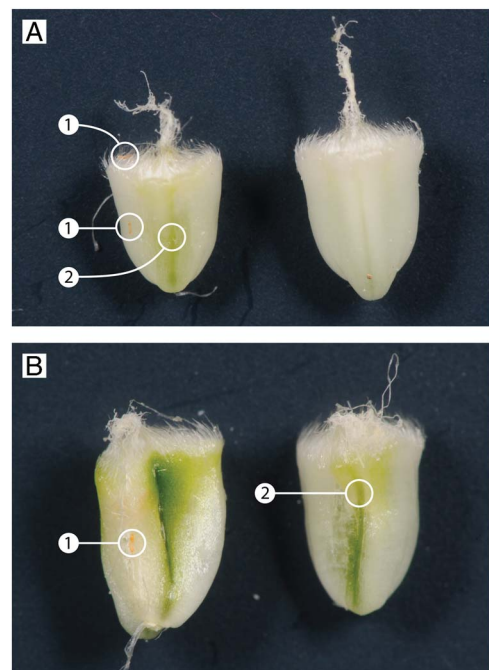
For each resistant cultivar, two or three seeds (8–11%) had visible feeding damage when examined within 12 days of exposure of the spikes (Fig. 2). No larvae were found at these damaged sites on the seed. The damage was most extensive on spikes exposed to midge at DS 0. Seed surfaces were collapsed, and were green and translucent above and below the feeding site (Fig. 2). Damaged seeds of Vesper and CDC Utmost were slightly more distorted than Shaw.

At maturity, seed of resistant cultivars damaged at an early development stage (DS 0–2) were often distorted dorsally or laterally (Fig. 3). The seeds were reduced in size but < 5% were damaged sufficiently to render them not harvestable compared with > 60% of damaged seed in the susceptible checks.

### Larvae per spike in laboratory tests

The number of third instars on the susceptible checks was highest in the first three spike stages and decreased thereafter in 2008, 2009, and 2012 (Fig. 1). Fewer third instars survived on spikes of susceptible cultivars when spikes were exposed to

**Fig. 2.** Seed damage eight days after the resistant cultivar Shaw (A) and 12 days after the resistant cultivar CDC Utmost (B) were exposed to adults of *Sitodiplosis mosellana* on the same day spikes emerged in laboratory tests. 1 = first instars; 2 = site of feeding damage.





the highest, and one spike of Vesper had seven larvae. Spike stage during oviposition did not influence third instar abundance on Fieldstar, as the five most infested spikes ranged in exposure time from DS 0–4. Eighteen of the 21 larvae on Vesper were found on spikes with DS 0–1 exposures and none after DS 3.

### Effect of stage of spike development on seed damage

The parameter estimates ( $a$  = % yield loss when spikes were exposed at DS 0, and  $b$  = the reduction in yield loss (%) when spikes were exposed at DS 0–5) for all cultivars from the tests in 2008 and 2009 and for the cultivars 2011 and 2012 did not differ ( $P > 0.05$ ). Thus, the data were pooled to form two separate tests. The relationship between the decrease in yield loss % ( $y$ ) relative to the age of spikes at exposure was higher for  $\log(y)$  than for  $y$  for all wheat entries, except Vesper in 2011/2012 ( $r^2 = 0.96$  versus  $r^2 = 0.99$ ) and for the checks in both pooled tests ( $r^2 = 0.87$  and  $0.94$  versus  $r^2 = 0.97$  and  $0.99$ ). Thus, results for all wheat are presented as log transformed data for ease of comparison (Fig. 4, Table 3).

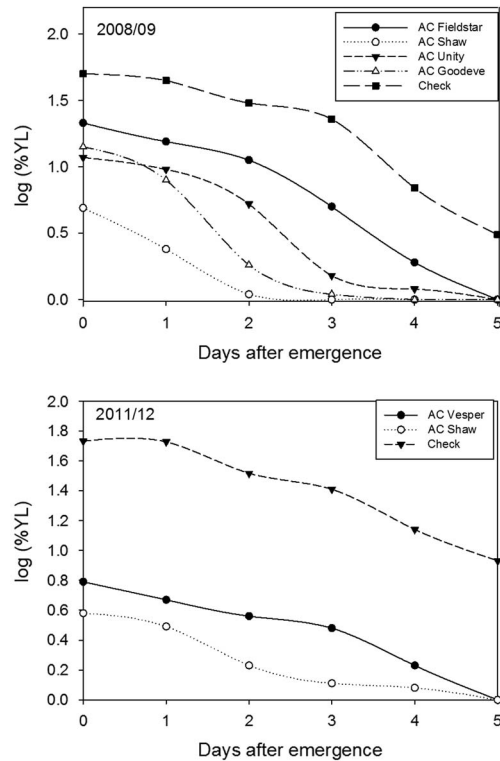
Yield losses by the midge decreased for all susceptible and resistant cultivars and advanced lines in laboratory tests ( $P < 0.05$ , least square means (LSM) Tukey-Kramer adjustment) with an increase in the age of the spikes (DS) when exposed to the midge (Fig. 4). The relationship between yield loss and age of spike when exposed to adult midge accounted for the vast majority of the decrease in yield loss ( $r^2 \geq 0.91$ ) among spikes for Fieldstar, Unity, Vesper, Shaw in 2011/2012, and the check in 2011/2012, and for most of the decrease ( $r^2 \geq 0.80$ ) for Goodeve, CDC Utmost, the check in 2008/2009, and BW314 (Table 3).

The effect of DS on yield loss ( $b$ ) was similar to the check for Fieldstar, Unity, Goodeve, Vesper, and BW314 (Table 3). A reduction in yield loss with DS was lower than the check for Shaw, CDC Utmost, and BW430. All three of these resistant wheats had similar reductions in yield loss with DS.

### Yield losses to resistant cultivars in laboratory tests

When compared to yield losses in all tests, excluding DS 5, each DS for resistant wheats had lower yield losses than the checks ( $P < 0.05$ , LSM, Tukey-Kramer adjustment). Yield losses for DS 5 did not differ because larvae largely

**Fig. 4.** Yield losses (%) by resistant wheat cultivars with *Sml* when exposed to adults of *Sitodiplosis mosellana* zero to five days (DS 0–5) after emergence of spikes in laboratory tests.



failed to establish on the seed of susceptible cultivars (Fig. 4). Thus, total yield loss assessments, in which yield losses per DS were given equal weight, only included spikes exposed at DS 0–4.

All resistant cultivars had lower ( $P < 0.01$ , LSM) total yield losses than the checks in all tests (Table 4). Shaw and BW430 had the lowest amount of feeding damage by *S. mosellana* (Table 4). Compared with other resistant cultivars, yield losses by midge for Shaw were significantly lower than for Fieldstar only. Goodeve also had lower yield losses than Fieldstar in 2008, but not in 2009. All other yield loss comparisons, excluding BW430, among the resistant cultivars did not differ ( $P > 0.05$ ).

### Yield losses in field tests

Only eight of the 18 field tests (six sites  $\times$  three years) grown in 2009–2011 had midge

**Table 3.** Estimates of yield loss (%) by resistant and susceptible wheat when spikes were exposed to *Sitodiplosis mosellana* adults zero to five days after emergence of spikes (DS 0–5) in laboratory tests, 2008–2012.

Wheat	Number of laboratory tests	$r^2$	$P$	$a^1$	$b^2$
Fieldstar	2	0.95	<0.001	$\log 1.452 \pm 0.083$	$\log 0.278 \pm 0.027$
Shaw	2	0.67	0.029	$\log 0.516 \pm 0.120$	$\log 0.132 \pm 0.040$
Unity	2	0.91	0.002	$\log 1.112 \pm 0.102$	$\log 0.246 \pm 0.034$
Goodeve	2	0.80	0.011	$\log 1.007 \pm 0.165$	$\log 0.247 \pm 0.054$
Check	2	0.87	0.004	$\log 1.867 \pm 0.125$	$\log 0.245 \pm 0.041$
BW314	1	0.80	0.011	$\log 0.974 \pm 0.161$	$\log 0.241 \pm 0.053$
BW430	1	0.72	0.021	$\log 0.446 \pm 0.090$	$\log 0.111 \pm 0.030$
Vesper	2	0.96	<0.001	$\log 0.831 \pm 0.042$	$\log 0.153 \pm 0.014$
Shaw	2	0.91	0.002	$\log 0.553 \pm 0.050$	$\log 0.121 \pm 0.017$
Check	2	0.94	<0.001	$\log 1.828 \pm 0.057$	$\log 0.167 \pm 0.019$
CDC Utmost	1	0.82	0.008	$\log 0.473 \pm 0.070$	$\log 0.112 \pm 0.023$

<sup>1</sup> Yield loss (%) by *S. mosellana* when spikes exposed on the same day of their emergence from the boot.

<sup>2</sup> Reduction in % yield loss =  $(\log a - \log bx) - 1$ , where x = days after spike emergence.

**Table 4.** Yield losses (% ± SE) by *Sitodiplosis mosellana* to the resistant component of spring wheat cultivar blends and to advanced lines in four laboratory tests from 2008 to 2012 when adults were exposed zero to four days after spikes emerged (DS 0–4).

Wheat	2008			2009			% of Check
	Cages	Spikes	Loss ± SE	Cages	Spikes	Loss ± SE	
Fieldstar	6	36	$7.3 \pm 0.7^{*\ddagger}$	9	53	$14.9 \pm 3.4^{*\ddagger}$	35.9
Shaw	5	33	$0.6 \pm 0.3^*$	9	60	$1.7 \pm 0.8^*$	3.7
Unity	5	25	$3.5 \pm 1.3^*$	10	86	$6.4 \pm 2.4^*$	16.0
Goodeve	6	29	$2.3 \pm 1.2^*$	6	59	$3.6 \pm 1.5^*$	9.5
Check	7	89	$26.5 \pm 1.5$	10	238	$35.3 \pm 5.3$	–
BW314	–	–	–	8	55	$6.0 \pm 2.4^*$	17.0
BW430	–	–	–	8	87	$0.9 \pm 0.3^*$	2.5
Wheat	2011			2012			% of Check
	Cages	Spikes	Loss ± SE	Cages	Spikes	Loss ± SE	
Vesper	5	98	$1.8 \pm 0.9^*$	7	90	$3.3 \pm 1.0^*$	7.5
Shaw	5	50	$1.5 \pm 0.6^*$	7	79	$0.9 \pm 0.4^*$	3.5
Check	5	46	$30.4 \pm 4.4$	7	78	$37.3 \pm 3.3$	–
CDC Utmost	–	–	–	7	81	$2.1 \pm 0.6^*$	5.6

\* $P$  (Least-squares means test with Tukey-Kramer adjustment) difference from check ( $P < 0.01$ ).

‡ $P$  (Least-squares means test with Tukey-Kramer adjustment) difference from Shaw ( $P < 0.01$ ).

infestations that reduced yields in the checks, and were included in the results. Yield losses were significantly less than AC Barrie for all resistant cultivars at all sites except for Goodeve at Outlook in 2009, where midge damage was the lowest among the eight tests (Table 5). Shaw had the least amount of damage of all wheat cultivars at every site (1.0% of AC Barrie). It also had significantly less damage to the resistant component of the

varietal blend than all other resistant wheat cultivars at one or more sites (Table 5).

Yield losses for Unity, Goodeve, CDC Utmost, and Vesper relative to susceptible wheat were 5.6–17.0% in the cage tests (Table 4) and 7.3–10.0% in the field (Table 5). Fieldstar yield losses were twice that of Unity and Vesper in the field and of Unity and Goodeve in cage tests (Tables 4, 5).



**Table 5.** Yield losses (%) by the resistant component of spring wheat cultivar blends and by AC Barrie to *Sitodiplosis mosellana* in field tests at Kernen (KRN), Outlook (OLK), Scott (SCT), and Indian Head (IH) Saskatchewan from 2009 to 2011.

Wheat	2009			2010		2011			% of AC Barrie
	KRN	OLK	SCT	KRN	IH	KRN	IH	SCT	
Fieldstar	3.9* <sup>‡</sup>	0.2*	3.0*	–	–	0.5*	3.0*	2.6*	15.1
Shaw	0.3*	0*	0.3*	0.3*	0.2*	0*	0.5*	0.2*	1.0
Unity	0.6*	0.1*	2.3*	2.7* <sup>‡</sup>	1.9*	0.2*	1.8*	2.5*	7.3
Goodeve	0.9*	0.6	2.2*	–	–	0.5*	1.3*	3.3* <sup>‡</sup>	10.0
CDC Utmost	1.6*	0.1*	1.9*	3.4* <sup>‡</sup>	1.4*	0.1*	1.2*	4.7* <sup>‡</sup>	8.5
AC Barrie	19.2	1.7	13.5	41.7	32.0	4.3	20.4	28.2	–
Vesper	–	–	–	2.6* <sup>‡</sup>	2.2*	0.3*	1.2*	3.2* <sup>‡</sup>	7.3

\**P* (Least-squares means test with Tukey-Kramer adjustment) difference from AC Barrie (*P* < 0.05).

<sup>‡</sup>*P* (Least-squares means test with Tukey-Kramer adjustment) difference from Shaw (*P* < 0.05).

## Discussion

The stage of spike development up to DS 5 had no effect on oviposition preference by females for all resistant and susceptible cultivars or advanced lines. This concurs with previous studies on susceptible wheat (Ding and Lamb 1999; Smith and Lamb 2001) and indirectly shows any subsequent lack of seed damage to resistant wheats at all spike stages in the study was a product of the inability of larvae to establish a feeding site on the seed and not because of an absence of larvae.

Oviposition deterrence is a heritable plant trait in susceptible and antibiotic *Sm1*-resistant spring wheat and has been identified in lines with different pedigrees (Lamb *et al.* 2000a, 2002; Gharalari *et al.* 2009). This study reports deterrence for the first time in an antibiotic commercial cultivar, Vesper (Table 1). Although this cultivar received significantly fewer eggs in the cage studies than Shaw, CDC Utmost or the check, it was no different than Unity, CDC Utmost, or Goodeve in yield losses in field tests, or than CDC Utmost in cage tests (Tables 4, 5). These results indicate that levels of oviposition deterrence expressed by Vesper may not always have direct additive benefits in preventing seed damage in *Sm1* resistant cultivars. However, by reducing larval selection pressure, oviposition deterrence could help to prolong *Sm1* efficacy in varietal blends.

The development of third instars on the seed of wheats with *Sm1* resistance in our laboratory studies allows for comparison with two previous

studies. Ding *et al.* (2000) found that plants deriving *Sm1* from Seneca wheat had higher larval mortality than those derived from Howell. This differs with our studies on the Howell-derived spring wheats Shaw, BW314, and BW430, and the Seneca-derived CDC Utmost (Table 2). This contrast, as well as differences observed in our studies with Clark-derived cultivars (Fieldstar, Unity, Goodeve), indicates that gene(s) linked to *Sm1* expression are not strongly expressed in winter wheat backgrounds. Smith *et al.* (2007) found that most third instars found on resistant wheat in cage studies were smaller than those on susceptible wheat, as we also observed. They were often found on late-aborted seed that provide just enough food for their survival. These larvae likely had abandoned their initial feeding site as first instars and had moved to the aborted seed, as was observed on resistant seed 8–12 days after oviposition.

Outside of a few dead second instars, no other larvae or cast skins were found on the seed of resistant cultivars in the field. Dead first instars on spikes by the time of the assessments would have decomposed. On comparable field tests, Smith *et al.* (2007) also reported no survival of third instars on “Key 97–24” (Monon-derived *Sm1*) resistant wheat. These results indicate the small third instars found on resistant wheat in the laboratory were a product of the optimal abiotic conditions created for midge survival.

The large difference in the number of third instars found on Shaw and Fieldstar (Table 2) suggests the inducement of resistance between

resistant cultivars is highly variable and is prevalent whether seeds fully form or prematurely abort. Ferulic and *p*-coumaric acid production by resistant wheats is induced by the presence of larvae on the seed (Ding *et al.* 2000), but not at levels that necessarily account for differences in resistance. Ding *et al.* (2000), however, did conclude that acid production was more pronounced between resistant and susceptible seed, and larval mortality was most associated with levels of ferulic acid of  $> 0.35 \mu\text{g/g}$  fresh weight on the surface of the seed. The speed at which a resistant cultivar can attain that fatal concentration is likely more important in deterring feeding and development of larvae than mean concentrations.

The age of the spikes (DS) for resistant wheats had no effect on larval survival. This differed from results on susceptible cultivars, where survival declined at DS 4–5. The resistant wheat data, however, was based on a small number of larvae on a low percentage of infested seed and applied almost exclusively to Fieldstar. Larvae observed on other more resistant cultivars, except for Shaw where none were found, were only on spikes of the first two exposure times (DS 0–1).

Cell maturation in susceptible cultivars was found to act as a partial resistant mechanism, whereby the ability of larvae to feed and, subsequently, survive and cause seed damage was reduced at later developmental stages (DS 4–5). On later resistant seed (DS 3–5), larvae also attempted to feed but were soon deterred by a hypersensitive immunologic reaction of acid induction as described earlier. Many of these seeds had no visible symptoms or had a slightly distorted shrunken appearance; first described by Barker and McKenzie (1996) as “tubby” seeds.

As indicated by the absence of oviposition deterrence, excluding Vesper, an equal number of first instars attempted to feed on the seed of both resistant and susceptible cultivars. Within the resistant lines, significant differences in the degree of damage to seeds were observed between resistant cultivars and between stages within the cultivars. Seed injury by larvae for resistant cultivars were reduced at later stages (DS 3–5), as seen for susceptible cultivars, compared with the first exposure. This appears to be a function of the immune response acting in concert with a decreasingly suitable seed surface for larval establishment. The difference in the prevention of

seed damage between resistant cultivars, therefore, is likely due solely to the strength of the hypersensitive response as opposed to cultivar differences in the suitability of the seed surface as a feeding site. Since larvae need to initiate feeding on the seed to determine its suitability as a food source, the speed of induction needs to be extremely rapid to prevent permanent cellular damage, particularly to cells of newly developing kernels and to prevent any possibility of larvae ingesting sufficient food to reach the third instar before feeding is deterred.

The variability in seed damage to resistant lines and to different stages of spike emergence are important considerations during the selection of wheat lines in breeding programmes. The winter wheat origin of *Sm1* does not appear to provide any advantage for the expression of antibiotic resistance. The presence of gene(s) that either directly or through improved agronomics enhance *Sm1* effectiveness requires investigation. At present, Shaw is the best midge-resistant cultivar for preventing seed damage by the midge, and, in future, this cultivar could serve as the resistant standard of excellence in the selection of cultivars with minimal yield or grade losses caused by *S. mosellana*.

## Acknowledgements

The authors wish to thank Sheila Wolfe, Thelma Czarnecki, Elaine Martineau, and Tesfu Araia of the Cereal Research Centre for technical assistance, and Blaine Recksiedler, Saskatchewan Agriculture and Food, for co-ordinating the seeding and collection of wheat samples at all field sites.

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