Seed damage and sources of yield loss by *Sitodiplosis mosellana* (Diptera: Cecidomyiidae) in resistant wheat varietal blends relative to susceptible wheat cultivars in western Canada

M.A.H. Smith,[1] I.L. Wise, S.L. Fox, C.L. Vera, R.M. DePauw, O.M. Lukow

Abstract—Spring wheat varieties with the *Sm1* gene for resistance to wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), were compared with susceptible wheat (*Triticum* Linnaeus; Poaceae) with respect to sources of yield loss and reduction in market value from wheat midge feeding damage. Four resistant varietal blends (90% *Sm1* wheat plus 10% susceptible refuge) and four susceptible cultivars were grown in replicated experiments at eight locations in western Canada. Frequencies and 1000-kernel weights of undamaged and midge-damaged seeds were assessed before harvest by dissecting samples of ripe spikes, and after harvest in samples of cleaned grain. Spike data were used to estimate yield losses from reduced weight of damaged seeds and loss of severely damaged seeds (≤8 mg) at harvest. Among midge-damaged seeds in spikes, few were severely damaged in resistant varietal blends, whereas most were severely damaged in susceptible cultivars. Cleaned, harvested grain of resistant varietal blends and susceptible cultivars had similar frequencies of midge damage and were assessed similar market grades. The primary benefit of midge-resistant wheat was reduced yield loss due to seed damage by wheat midge larvae. Resistant wheat did not protect against loss of market grade, but market value could increase due to larger yields.

Résumé—Nous avons comparé des variétés de blé de printemps possédant le gène *Sm1* de la résistance à la cécidomyie du blé, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), à du blé (*Triticum* Linnaeus; Poaceae) vulnérable en ce qui a trait aux sources de perte de rendement et à la réduction de la valeur marchande à cause des dommages causés par l'alimentation de la cécidomyie du blé. Quatre mélanges de variétés résistantes (90% de blé *Sm1* plus 10% de blé vulnérable comme refuge) et quatre cultivars vulnérables ont été cultivés lors d'expériences menées en double dans huit sites de l'Ouest canadien. Nous avons évalué les fréquences et les masses de 1000 épis de grains sains et de grains endommagés par les cécidomyies avant la récolte en disséquant des échantillons d'épis mûrs et, après la récolte, en mesurant des échantillons de grains propres. Les données provenant des épis ont servi à estimer les pertes de rendement dues à la masse réduite des grains endommagés et la perte de grains fortement endommagés (≤8 mg) lors de la récolte. Parmi les grains endommagés dans les épis par les cécidomyies, peu de grains étaient fortement endommagés chez les mélanges de variétés résistantes, alors que la plupart des grains étaient fortement endommagés chez les cultivars vulnérables. Les grains récoltés et nettoyés des mélanges de variétés résistantes et des cultivars susceptibles montraient des fréquences semblables de dommages dus aux cécidomyies et ils ont obtenu des cotes marchandes semblables. L'avantage principal du blé résistant aux cécidomyies est la réduction des pertes de rendement causées par les dommages dus aux larves de cécidomyies du blé. Le blé résistant n'offre pas de protection contre la perte de cote marchande, mais sa valeur marchande peut augmenter à cause de ses rendements plus élevés.

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Introduction

The orange wheat blossom midge (here referred to as wheat midge), *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), is a serious insect pest of spring wheat (*Triticum* Linnaeus; Poaceae) in western Canada (Olfert *et al.* 1985; Lamb *et al.* 1999). Feeding by larvae on newly developing wheat seeds can either completely destroy the seed or result in the presence of partially damaged seeds at harvest, contributing to yield loss, reduction in end-use suitability (Dexter *et al.* 1987), and market grade as well as reduced germination and growth rate of newly germinated seedlings (Lamb *et al.* 2000).

The wheat midge is distributed throughout all major wheat-growing areas of western Canada, causing some seed damage in most areas each year and causing extensive yield losses during population outbreaks in Saskatchewan, Canada in the early to mid-1980s (Olfert *et al.* 1985; Olfert *et al.* 2009) and Manitoba, Canada in the early to mid-1990s (Lamb *et al.* 1999). Midge populations can be controlled using insecticides (Elliott 1988), but timing of application is problematic because of the narrow range of available dates for control. Adult females are active and oviposit only at dusk while the wheat crop is heading and are followed a few days later by their egg parasitoid, *Macroglenes penetrans* (Kirby) (Hymenoptera: Pteromalidae), which is also susceptible to the same insecticides.

The opportunity to manage the wheat midge by host-plant resistance became possible with the discovery of the *Sm1* gene in winter wheat (McKenzie *et al.* 2002), conferring antibiotic resistance to newly hatched wheat midge larvae, which stop feeding soon after reaching the newly developing seeds. The *Sm1* gene has been incorporated into the major classes of spring wheat grown in western Canada (Vera *et al.* 2013). Midge-resistant spring wheat cultivars are now sold commercially in Canada as varietal blends of 90% midge-resistant wheat and 10% susceptible wheat. These blends produce an interspersed refuge to manage the potential development of virulent midge biotypes, which could survive on resistant wheat, while at the same time preserving parasitoid populations (Smith *et al.* 2004, 2007). Resistant spring wheat cultivars are believed to be the first field crops to incorporate a refuge as a varietal blend for insect population management. Cotton and corn that incorporate Bt toxins use refuges as separately planted fields, but recently the United States Environmental Protection Agency approved the registration of blends of Bt corn and non-Bt corn (Onstad *et al.* 2011) and have been used for the past three years to manage Bt-resistant insects (Coghlan 2013). Resistance to insect pests based on a single gene, such as *Sm1*, often is short-lived because of the selection of virulent insect biotypes (Harris *et al.* 2003). A 10% refuge produces enough avirulent adults to discourage the selection of virulence, especially if the inheritance of the resistance is recessive and the heterozygous offspring cannot survive on resistant wheat plants (Tabashnik *et al.* 2008).

The *Sm1* gene does not interact negatively with the agronomic characteristics of the resistant spring wheats in which it is incorporated (Vera *et al.* 2013). Resistant varietal blends yield at least as much grain as conventional susceptible cultivars in the absence of midge damage, and yields are much higher than susceptible cultivars when moderate to high midge damage occurs. There are several sources of yield loss due to wheat midge damage, including reduced weight of damaged seeds, loss of severely damaged seeds, and loss of small seeds when the grain is cleaned; in addition, visible midge damage in inspected grain can result in loss of market value (Lamb *et al.* 2000). The objective of this study was to quantify these sources and evaluate their importance in reducing yield loss and preserving market grade in midge-resistant varietal blends in comparison with susceptible cultivars.

Materials and methods

The study was conducted from 2007 to 2010 at eight locations in western Canada (Brandon, Manitoba; Indian Head, Regina, Saskatoon, Melfort, and Swift Current, Saskatchewan; Lethbridge and Lacombe, Alberta), representing areas varying from very-low-to-high expected wheat midge densities (Vera *et al.* 2013). For each location and year, the experiment consisted of a randomised complete block design with four replications. Treatments were four wheat midge resistant spring wheat cultivars having the

Sm1 gene and including a 10% susceptible refuge (varietal blends), and four susceptible check cultivars. Varietal blends were Goodeve VB (90% Goodeve and 10% AC Intrepid), Fieldstar VB (90% Fieldstar and 10% Waskada), Shaw VB (90% Shaw and 10% BA51*C222), and Unity VB (90% Unity and 10% Waskada). Susceptible check cultivars were AC Intrepid, CDC Teal, Katepwa, and Waskada (see Vera *et al.* (2013) for wheat cultivar references).

Seed of the varietal blends was reconstituted annually from a common seed source to guarantee a 90:10 seed ratio at all sites each year. Plot size varied by location from 2.7 to 3.7 m^2, and were seeded in May, except in Saskatoon in 2007 (5 June) and 2010 (13 June) because of wet fields, at a seeding rate of 220 viable seeds per m^2. Field sites were fertilised according to recommendations following soil tests (Vera *et al.* 2013).

Each year, when kernels were at the hard dough stage (Zadoks growth stage 87; Tottman and Makepeace 1979), 12–20 wheat spikes were collected randomly from all plots at each location and air dried at room temperature. Ten spikes per plot were dissected under a stereomicroscope and numbers of undamaged seeds (= not midge damaged), harvestable midge-damaged seeds (individual weights >8 mg), and unharvestable midge-damaged seeds (⩽8 mg) were recorded for each spike. Determination of the categories was based on previous studies of effect of wheat midge damage on seed weights, where seeds weighing ⩽8 mg were lost with the chaff during combining (Lamb *et al.* 2000). The great majority of seeds could be sorted visually, with very few needing to be individually weighed.

Two measures of the proportion of midge-damaged seed for each spike sample were calculated: the proportion of harvestable damaged seed among all harvestable seed (undamaged + harvestable damaged), and proportion of all damaged seeds among all seed (undamaged + harvestable damaged + unharvestable damaged). To obtain seed weights, seed from each 10-spike sample was pooled by damage category, then undamaged and harvestable damaged seed weights were obtained to the nearest milligram, then standardised by calculating 1000-kernel weights for each sample of undamaged and harvestable damaged seed.

The proportion of yield lost due to wheat midge damage was estimated based on the number of unharvestable seeds lost (pooled over 10 spikes) and the weight reduction of harvestable damaged seeds. Proportion of yield lost from harvestable damaged seeds was based on the difference between seed weights of undamaged and harvestable damaged seeds, which was then converted to an equivalent number of undamaged seeds:

$$\text{Proportion of seed weight lost} = ((undam_{kwt} - dam_{kwt}) / undam_{kwt}) \times ndam \qquad [1]$$

where $undam_{kwt}$ and dam_{kwt} are 1000-kernel weights of undamaged and harvestable damaged seed, respectively, and *ndam* is number of harvestable damaged seeds. Then weight lost due to unharvestable seeds was added to the result of equation [1] to find percentage yield loss:

$$\%\,\text{yield loss} = ([\text{equation 1}] + nunharv) / \text{total number of seeds}) \times 100 \qquad [2]$$

where *nunharv* is number of unharvestable seeds in a 10-spike sample. Percentage yield loss was calculated for each plot sample in each location and year.

Seed from each plot was harvested at crop maturity with a small plot combine, and then cleaned to remove chaff and very small seeds (Vera *et al.* 2013). A 50-g seed sample from each plot was sent to grain inspectors at the Canadian Grain Commission (CGC) in Winnipeg, Manitoba, Canada for a visual assessment of percentage of wheat midge seed damage. Each year the same grain inspector assessed all samples. The samples were then sent to Agriculture and Agri-Food Canada's Cereal Research Centre in Winnipeg, Manitoba, Canada for assessment of midge damage under a stereomicroscope in a laboratory. The two damage assessments were referred to as "estimate" and "actual", respectively. At least 1000 seeds per 50-g sample were examined and categorised as undamaged and midge damaged. Broken seeds and seeds with another degrading factor that prevented determination of wheat midge injury were not counted. Undamaged and midge-damaged seed samples were weighed and 1000-kernel weights calculated as for spike samples.

Statistical analysis

All data were analysed using procedures of SAS® (SAS Institute 2008). For all tests, differences were considered significant at $P \leqslant 0.05$. Before analyses of variance, data distributions were examined and data were checked for relationships between variances and means. Proportions of midge-damaged seed were transformed using arcsine $\sqrt{x}$. Percentage yield loss was square-root transformed. None of the seed weight data required transformation. For calculation of means and analyses of variance, numbers of seeds in the samples were used as weighting factors for proportions of damaged seed and 1000-kernel weights. For the analysis of covariance of 1000-kernel weights of midge-damaged seed, the number of damaged seeds was used as a weighting factor. For all mixed-model analyses of variance, denominator degrees of freedom were estimated using the Satterthwaite method. The eight treatments were referred to collectively as cultivars.

Variation in the proportion of midge-damaged seed was assessed at several levels: (1) among all seed from dissected spikes; (2) among harvestable seed from dissected spikes, excluding unharvestable seed; (3) among cleaned, harvested seed estimated at the CGC, and then assessed under a microscope in a laboratory. To compare proportions of midge-damaged seed between resistance classes (resistant varietal blends and susceptible cultivars), each analysis was performed using PROC MIXED, with resistance class and cultivar within resistance class as the fixed effects and location, resistance class × location and replicate within location as random effects. Comparison of the two assessments of the harvested grain samples (estimate and actual) was performed as a split-plot mixed-model analysis of variance, as above, with assessment method as the subplot factor.

The 1000-kernel weights of harvestable damaged seed from dissected spikes and cleaned, harvested grain samples were subjected to an analysis of covariance, with 1000-kernel weights of undamaged seed used as the covariate. The fixed effects were resistance class and cultivar within resistance class and random effects were location, resistance class × location and replicate within location.

The two independent measures (from dissected spikes and harvested grain samples) of percentage of harvestable damaged seed and of 1000-kernel weights of harvestable damaged seed were examined for consistency using a split-plot analysis of variance using PROC MIXED. Cultivar, sampling method (dissected spike samples and cleaned, harvested grain samples) and their interaction were fixed effects, with sampling method as the subplot factor. Location, replicate within location and cultivar × replicate within location were random effects.

Results

Seed damaged by wheat midge was detected in all treatments at most locations in all years, but varied highly, with mean total damage of 13.7% (range: 0–50.7), 1.8% (0–8.3), 2.3% (0–10.3), and 6.4% (0–30.8) in 2007, 2008, 2009, and 2010, respectively. In general, resistant varietal blends had less midge-damaged seed than susceptible cultivars. Detection of differences in degree of damage between resistant varietal blends and susceptible cultivars depended on how percentage damage was measured (Fig. 1), but within each resistance class (resistant varietal blends and susceptible cultivars) there were always highly significant differences among cultivars (Table 1). Within the resistant varietal blends, Shaw VB always had the least damage and within the susceptible cultivars Waskada always had the least (Fig. 1) in all four years in spike samples and in harvested grain samples.

When midge damage was measured by dissecting ripe spikes to observe all seed, resistant varietal blends always had much less total damage than susceptible cultivars (Table 1). When unharvestable seeds ($\leqslant$8 mg) were excluded, small differences (in 2008) or no differences were detected between the two resistance classes of wheat in the proportion of damaged seed (Table 1). Unharvestable seed accounted for averages of 3–46% of damaged seed in resistant varietal blends and 55–71% of damaged seed in susceptibles, over the four years.

When actual percentage of midge damage in samples of cleaned, harvested grain was assessed using a microscope, differences between resistant varietal blends and susceptible cultivars were highly significant in 2008 and 2009, but undetectable in 2007 and weakly significant in 2010 ($P = 0.025$; Table 1). Comparison of percentage of midge-damaged seed in harvestable seed from

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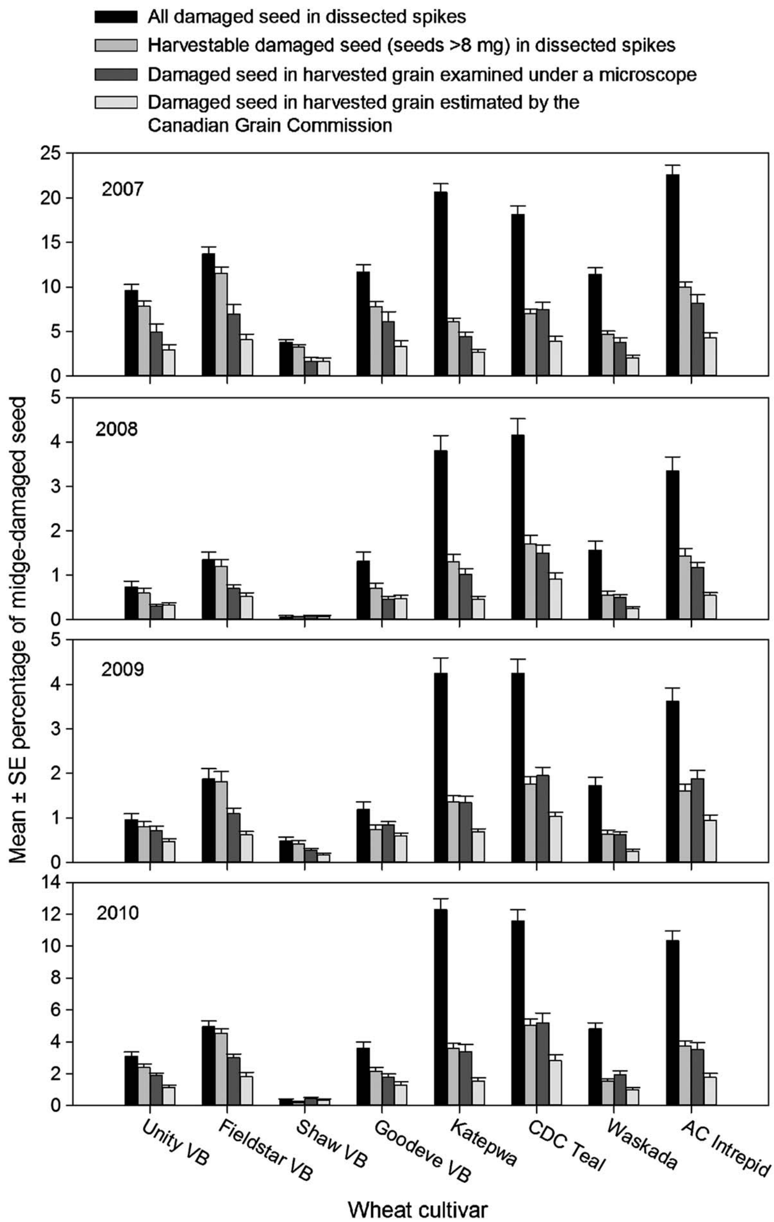
Fig. 1. Means ± SE of four measures of wheat-seed damage by *Sitodiplosis mosellana* to midge-resistant varietal blends (VB; contain a 10% susceptible refuge) and susceptible cultivars. Midge damage to each experimental plot was assessed in samples of dissected ripe spikes and in cleaned, harvested grain.



spikes versus harvested grain showed that the two sampling methods produced similar results in 2008 and 2009. However, in 2007 and 2010 midge damage was generally greater in harvestable seed from spikes than in harvested grain (Table 2). When harvested grain samples were assessed visually by CGC grain inspectors to estimate midge damage, differences between the two resistance classes were weakly significant (2008: $P = 0.042$; 2009: $P = 0.045$) or undetectable (2007 and 2010; Table 1). The percentage of midge-damaged seed detected by visual

Table 1. Analysis of variance (ANOVA) and covariance (ANCOVA) (MIXED procedure) of effect of resistance class and wheat cultivar within resistance class on the percentage of seed damaged by *Sitodiplosis mosellana* and 1000-kernel weights of damaged seed.

Dependent variable and fixed effects	2007	2008	2009	2010
% midge-damaged seed (ANOVA)				
In all seed from spike samples				
Resistance class	$F_{1,6} = 36.5$***	$F_{1,5.03} = 21.9$**	$F_{1,6.97} = 13.2$**	$F_{1,6} = 17.2$**
Cultivar (resistance class)	$F_{6,2200} = 72.9$***	$F_{6,1884} = 19.0$***	$F_{6,2490} = 20.9$***	$F_{6,2203} = 48.8$***
In harvestable seed from spike samples[†]				
Resistance class	$F_{1,5.96} = 0.02$ ns	$F_{1,5.04} = 7.95$*	$F_{1,7.09} = 5.14$ ns	$F_{1,6.01} = 2.74$ ns
Cultivar (resistance class)	$F_{6,2200} = 62.0$***	$F_{6,1884} = 14.5$***	$F_{6,2509} = 17.6$***	$F_{6,2220} = 49.8$***
Actual damage in seed from harvested grain				
Resistance class	$F_{1,6.03} = 3.96$ ns	$F_{1,4.92} = 52.4$***	$F_{1,7.02} = 27.0$***	$F_{1,7.01} = 8.00$*
Cultivar (resistance class)	$F_{6,204} = 48.6$***	$F_{6,152} = 36.1$***	$F_{6,210} = 53.2$***	$F_{6,210} = 87.4$***
CGC estimate from seed in harvested grain				
Resistance class	$F_{1,6} = 2.01$ ns	$F_{1,5.02} = 7.34$*	$F_{1,7} = 5.96$*	$F_{1,7} = 5.48$ ns
Cultivar (resistance class)	$F_{6,183} = 36.5$***	$F_{6,155} = 20.5$***	$F_{6,234} = 30.1$***	$F_{6,210} = 35.5$***
1000-kernel weight of damaged seed (ANCOVA)				
In harvestable seed from spike samples				
Resistance class	$F_{1,7.77} = 0.05$ ns	$F_{1,41.6} = 0.14$ ns	$F_{1,4.01} = 17.1$*	$F_{1,18.1} = 0.74$ ns
Cultivar (resistance class)	$F_{6,177} = 14.4$***	$F_{6,160} = 3.34$**	$F_{6,127} = 2.28$*	$F_{6,181} = 0.21$ ns
In harvested grain:				
Resistance class	$F_{1,12.4} = 0.05$ ns	$F_{1,7.67} = 8.27$*	$F_{1,14.4} = 0.20$ ns	$F_{1,225} = 11.2$***
Cultivar (resistance class)	$F_{6,194} = 5.37$***	$F_{6,160} = 0.53$ ns	$F_{6,232} = 3.12$**	$F_{6,222} = 2.65$*

Resistance classes were varietal blends (midge-resistant wheat with a 10% susceptible refuge) and midge-susceptible wheat. Dependent variables were measured in samples from each plot using two methods: dissecting a sample of ripe spikes and sampling cleaned, harvested grain. Midge damage in harvested grain was estimated by grain inspectors at the CGC and then assessed under a microscope in the laboratory (actual damage).

[†] "Harvestable" seeds weight >8 mg; smaller seed is lost at harvest.

*$P < 0.5$, **$P < 0.01$, ***$P < 0.001$.

ns, not significant; CGC, Canadian Grain Commission.

Table 2. Split-plot analysis of variance (MIXED procedure) to compare two sampling methods (sub-plots) used to measure seed damage by *Sitodiplosis mosellana* and 1000-kernel weights of midge-resistant and midge-susceptible wheat cultivars.

Dependent variable and fixed effects	2007	2008	2009	2010
% midge-damaged seed from independent samples				
Cultivar	$F_{7,237} = 37.6$***	$F_{7,359} = 43.2$***	$F_{7,486} = 36.2$***	$F_{7,300} = 58.6$***
Sampling method	$F_{1,220} = 78.1$***	$F_{1,359} = 2.94$ ns	$F_{1,486} = 2.61$ ns	$F_{1,217} = 4.67$*
Interaction	$F_{7,220} = 3.66$***	$F_{7,359} = 0.56$ ns	$F_{7,486} = 1.05$ ns	$F_{7,217} = 2.57$*
1000-kernel weights from independent samples				
Undamaged seed				
Cultivar	$F_{7,257} = 80.5$***	$F_{7,256} = 54.8$***	$F_{7,452} = 24.7$***	$F_{7,465} = 40.9$***
Sampling method	$F_{1,256} = 23.5$***	$F_{1,205} = 12.7$***	$F_{1,455} = 151$***	$F_{1,465} = 134$***
Interaction	$F_{7,255} = 3.01$**	$F_{7,219} = 1.74$ ns	$F_{7,452} = 0.30$ ns	$F_{7,465} = 1.01$ ns
Midge-damaged seed				
Cultivar	$F_{7,194} = 28.7$***	$F_{7,152} = 12.2$***	$F_{7,230} = 6.58$***	$F_{7,215} = 20.6$***
Sampling method	$F_{1,286} = 0.06$ ns	$F_{1,211} = 4.30$*	$F_{1,238} = 14.6$***	$F_{1,288} = 87.4$***
Interaction	$F_{7,290} = 5.06$***	$F_{7,217} = 0.77$ ns	$F_{7,245} = 3.96$***	$F_{7,295} = 0.44$ ns
% midge-damaged seed in harvested grain determined by independent assessment methods				
Resistance class	$F_{1,48} = 22.6$***	$F_{1,40.8} = 81.4$***	$F_{1,55} = 72.8$***	$F_{1,55} = 42.9$***
Assessment method	$F_{1,390} = 89.2$***	$F_{1,330} = 28.8$***	$F_{1,446} = 98.8$***	$F_{1,446} = 118$***
Interaction	$F_{1,390} = 4.72$*	$F_{1,330} = 22.1$***	$F_{1,446} = 11.1$***	$F_{1,446} = 5.89$*

Sampling methods of each plot were dissection of a sample of ripe spikes and assessment of a sample of cleaned, harvested grain. Midge damage to the harvested grain sample was estimated at the CGC and then assessed under a microscope in the laboratory.

*$P < 0.5$, **$P < 0.01$, ***$P < 0.001$.

ns, not significant; CGC, Canadian Grain Commission.

assessment was significantly less than the actual level of damage seen under a microscope in all years, but there was also an interaction with resistance class (Table 2). Estimated damage based on visual inspection detected ~60% and 50% of actual damage in resistant varietal blends and susceptible cultivars, respectively. Compared with total seed damage as measured in dissected ripe spikes, ~30–40% and 20% of midge-damaged seed in resistant varietal blends and susceptible cultivars, respectively, was detected by the visual assessments of the CGC grain inspectors.

The CGC estimates of midge damage were compared with the tolerance limit of 2% damage for wheat to be graded No. 1. All estimates, averaged over locations, were <2% in 2008 and 2009 and most were <2% in 2010, whereas most estimates were >2% in 2007 (Fig. 2). Of the varietal blends, Shaw VB always had <2% damage except at two locations in 2007, and Fieldstar VB had the greatest damage, with estimates >2% at six locations in 2007 and three locations in 2010. Of the susceptible cultivars, Waskada had the least damage, with estimates >2% at three locations in 2007 and one location in 2010. The other susceptible cultivars had damage estimates >2% at six locations in 2007 and three or four locations in 2010.

Weights of midge-damaged seeds in samples from dissected ripe spikes and from harvested grain were generally lower in the resistant varietal blends than the susceptible cultivars; however, the same pattern was observed in undamaged seeds (Table 3). When 1000-kernel weights of damaged seeds were adjusted for weight of undamaged seed in analysis of covariance, seed weights did not differ between resistant varietal blends and susceptible cultivars in three of the four years for harvestable seed from spikes and in two of the four years for harvested grain (Table 1). Comparison of 1000-kernel weights of damaged seed from spikes versus harvested grain showed sampling method to be not significant in 2007, but in the other years seed from spike samples weighed less than seed in harvested grain, except for Shaw VB and Goodeve VB in 2009 (Table 2). Sampling

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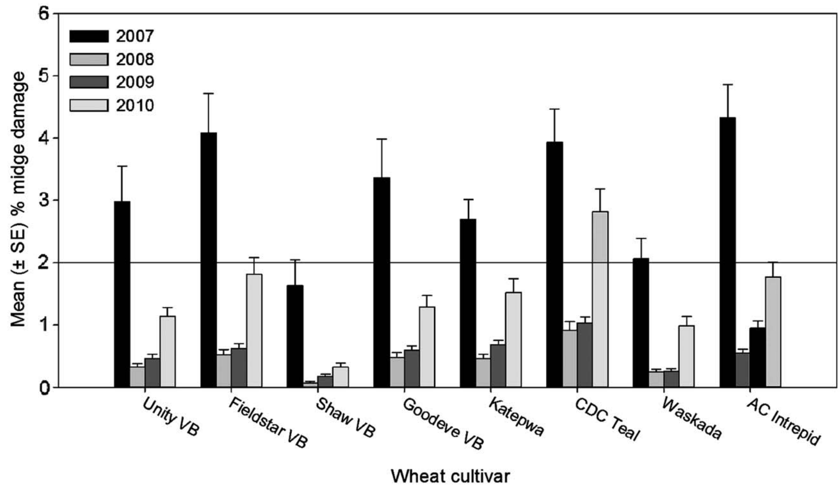
Fig. 2. Mean ± SE percentage of wheat seed damaged by *Sitodiplosis mosellana* in experimental plots of resistant varietal blends (VB; contain a 10% susceptible refuge) and susceptible cultivars. Seed damage in samples of cleaned, harvested grain from each plot was estimated by grain inspectors at the Canadian Grain Commission (CGC). The horizontal reference line at 2% indicates the midge-damage tolerance limit for wheat to be graded as No. 1.



method was a significant factor in undamaged seed-weight differences in all four years (Table 2). Generally, 1000-kernel weights of undamaged seed from spikes were 1–3 g less than seed from harvested samples (Table 3). Compared with weights of undamaged seed, damaged seed weights were generally 50–60% less in all years for both resistance classes and both sampling methods (Table 3).

Percentage yield loss (Fig. 3), estimated from number of unharvestable seeds and reduced weights of damaged seed in spikes, differed between resistant varietal blends and susceptible cultivars in all years in analyses of variance between resistance classes (2007: $F_{1,6} = 43.6$, $P = 0.0006$; 2008: $F_{1,5} = 33.4$, $P = 0.0022$; 2009: $F_{1,6.98} = 18.7$, $P = 0.0035$; 2010: $F_{1,6} = 43.5$, $P = 0.0006$). In each of the four years, the four resistant varietal blends had lower sample means than the four susceptible cultivars (Fig. 3). Among the resistant blends, Shaw VB always had the lowest yield loss and Unity VB the second lowest; among the susceptible cultivars, Waskada always had the lowest yield loss.

Discussion

This study showed that spring wheat resistant to *S. mosellana* received less seed damage and yield loss than conventional susceptible spring wheat, which did not have the *Sm1* gene for midge resistance, but the detection of these differences depended on when and how seed damage was measured. The greatest difference between the resistant varietal blends and susceptible cultivars was in yield loss from unharvestable seeds. When ripe wheat spikes were dissected to reveal all seeds, a much larger proportion of damaged seeds <8 mg in weight were found in susceptible spikes compared with resistant spikes. Severely damaged seeds weighing <8 mg would be blown with the chaff out the back of a combine during harvest and not seen in harvested grain (Lamb *et al.* 2000). There were damaged seeds found in wheat spikes of resistant varietal blends but most of these seeds were large enough to be retained in the harvested grain because nearly all wheat midge larvae would have stopped feeding soon after reaching the seeds (Ding *et al.* 2000). Much of the unharvestable seed seen in spike samples from varietal blends was observed to be concentrated in one or a few spikes per sample rather than distributed throughout the spikes, suggesting that most of the unharvestable seed was from spikes of the susceptible refuge component of the blends.

After unharvestable seed was removed from the samples of seed from dissected ripe spikes,

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Table 3. Mean ± SE 1000-kernel weights of wheat from experimental plots exposed to *Sitodiplosis mosellana* and measured in seed from samples of dissected ripe spikes and from cleaned, harvested grain.

		1000-kernel weights of seed (g)				Ratio of damaged to undamaged seed weights	
		Dissected spikes		Harvested grain			
Year	Resistance class	Undamaged	Harvestable damaged*	Undamaged	Damaged	Dissected spikes	Harvested grain
2007	Varietal blends†	30.7 ± 0.5	18.3 ± 0.3	31.5 ± 0.5	19.4 ± 0.2	0.60	0.62
	Susceptibles	32.2 ± 0.5	19.8 ± 0.3	32.8 ± 0.5	20.1 ± 0.2	0.62	0.61
2008	Varietal blends	34.0 ± 0.3	17.2 ± 0.4	34.7 ± 0.4	18.3 ± 0.3	0.51	0.53
	Susceptibles	35.9 ± 0.3	19.8 ± 0.4	36.0 ± 0.4	22.2 ± 0.4	0.55	0.62
2009	Varietal blends	33.7 ± 0.4	19.8 ± 0.5	37.0 ± 0.2	20.7 ± 0.3	0.59	0.56
	Susceptibles	34.8 ± 0.4	18.5 ± 0.4	38.3 ± 0.3	22.7 ± 0.3	0.53	0.59
2010	Varietal blends	31.1 ± 0.4	15.6 ± 0.3	33.9 ± 0.3	18.7 ± 0.2	0.50	0.55
	Susceptibles	32.7 ± 0.4	17.5 ± 0.3	35.4 ± 0.4	20.8 ± 0.2	0.54	0.59

*"Harvestable" seeds weight >8 mg; smaller seed is lost at harvest.
†Midge-resistant wheat with a 10% susceptible refuge.

differences between levels of midge-damaged seed in varietal blends and susceptible cultivars were small or undetectable. Similarly, when grain inspectors at the CGC examined harvested grain, differences between the two resistance classes were largely undetectable. However, when actual damage in harvested grain was examined under a microscope, differences were seen in 2008 and 2009, the two years with very low overall midge damage. In each of these three measures of midge damage, the mean for varietal blends was consistently lower than the mean for susceptible cultivars in each of the four years, yet most of the differences were not statistically significant. One reason for this may have been the differences among cultivars within each resistance class, resulting in high levels of variation within classes, discussed further below. Nevertheless, once unharvestable seed was removed from the samples either manually from dissected spikes or mechanically from harvested grain, midge-resistant varietal blends appeared to have little if any advantage over conventional susceptible cultivars. Some of the yield loss differences between resistance classes may have occurred if plants compensate for midge damage by diverting nutrients to undamaged seeds, but previous evidence does not support this hypothesis (Lamb *et al.* 2000).

Resistant varietal blends clearly have benefits to grain farmers. Overall midge damage is reduced considerably, but to identify this benefit wheat seeds in ripe spikes must be examined before the severely damaged seeds are lost at harvest. The greatest benefit of midge resistance was the retention of harvestable seed, which translated to increased yield. In 2007 and 2010 when midge damage was moderate to high, yield losses in varietal blends averaged 5% and 2%, respectively, while losses in susceptible cultivars averaged 14% and 8%, respectively. Even when midge damage was low in 2008 and 2009, varietal blends had yield losses of <1%, whereas susceptible cultivars experienced losses of between 2% and 3%. Vera *et al.* (2013) showed that midge resistance in varietal blends provided a yield advantage of about 10% over susceptible cultivars when midge damage was high (13% damage). Larger yields in varietal blends would mean greater market value; however, the resistant wheat was graded similar to the susceptible wheat because

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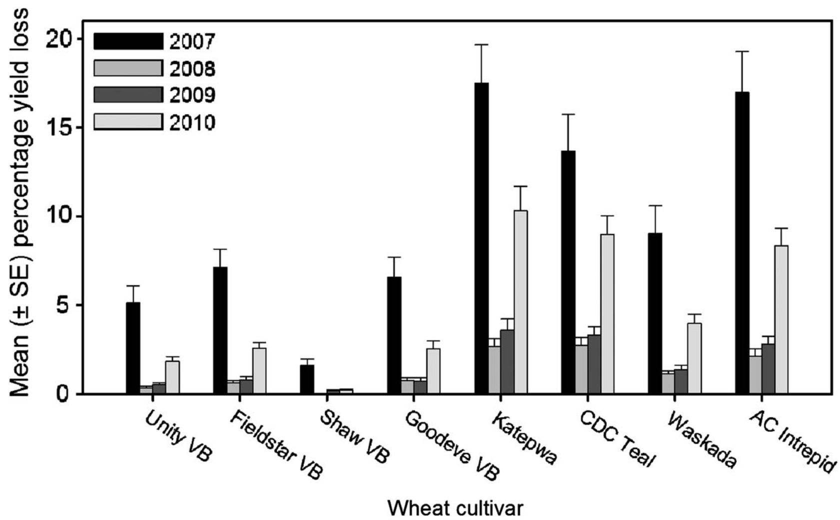
Fig. 3. Mean ± SE percentage yield loss due to wheat-seed damage by *Sitodiplosis mosellana* in experimental plots of resistant varietal blends (VB; contain a 10% susceptible refuge) and susceptible cultivars. See text for estimation of yield loss based on percentage midge-damaged seed and seed weights from dissected ripe spike samples.



similar levels of midge damage were detected in the harvested grain. Even though the *Sm1* resistance gene did not appear to contribute to protection of market grade, the improved yield would contribute to overall market value provided midge damage was low enough that the grain was not downgraded.

The four varietal blends showed variable responses to midge damage, but the consistency of the relative amounts of damage to the four blends suggests that genetic background of the wheat, rather than environmental effects, influenced the expression of the *Sm1* gene. Shaw VB had substantially less midge damage than the other three blends in all years. On the other hand, Fieldstar VB generally had greater midge damage compared with the other blends but a greater proportion of the damaged seed was harvestable. However, the resistant cultivar Fieldstar has smaller seeds than most other cultivars (Fox *et al.* 2012) and much of the harvestable damaged seed of the varietal blend has been shown to be lost as dockage when the seed is cleaned after harvest (Vera *et al.* 2013). These findings were not unexpected, as other studies have shown the expression of the *Sm1* gene to vary among wheat genotypes in which it has been incorporated (McKenzie *et al.* 2002).

The susceptible cultivar Waskada was less damaged than the other three susceptible cultivars according to all measures of midge damage. Unlike the resistant varietal blends, however, the proportion of midge-damaged seed that was unharvestable was similar to the other susceptible cultivars, with over half of the damaged seed being lost at harvest. Midge larvae develop normally on Waskada and severely damage many developing seeds, but there are fewer larvae because this cultivar deters ovipositing wheat midge females (Fox *et al.* 2009). Oviposition deterrence, a second type of resistance to wheat midge, is also inherited in spring wheat (Gharalari *et al.* 2009). Even though Waskada had a high proportion of severely damaged seeds, overall yield loss was lower, so compared with the other susceptible cultivars Waskada was downgraded by grain inspectors at fewer locations. In small research plots, midges may exercise genotype selection that is not apparent in large production fields of uniform genotypes. However, evidence from no-choice laboratory experiments suggests that oviposition deterrence may have value on a commercial scale (Lamb *et al.* 2002).

Yield losses were estimated from ripe wheat spikes collected before harvest and were based on seeds lost at harvest and reduced weight of harvestable seed. However, the measurement of seed weight was affected by sampling method, suggesting that these yield losses may be biased.

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Weights of harvestable seed from spikes were less than those of harvested, cleaned samples in three of the four study years. This difference could occur if "unharvestable seed" was incorrectly defined, and some small seeds weighing >8 mg were unharvestable. Lamb *et al.* (2000) obtained the 8 mg cutoff for unharvestable seed from both dissected spikes and commercial grain samples from railcars, and had agreement on seed sizes. The use of their definition of unharvestable seed is based on the premise that the railcar samples in Lamb *et al.* (2000) and our harvested plot samples were cleaned using the same screen sizes to remove dockage, which may not have been the case. Another possibility is that one or more of the individuals who dissected the spike samples in this study may have included seed smaller than 8 mg as "harvestable". Such a bias in measurement would result in a more conservative yield loss estimate.

This study demonstrated that the primary value of *Sm1*, conferring antibiosis against *S. mosellana* in resistant wheat, was reduced seed damage due to larval feeding, particularly the frequency of severely damaged seeds and resulting yield losses. To detect this benefit it was necessary to examine seed in spikes because severely damaged seeds are not retained at harvest. Once the grain was combine harvested and cleaned, frequencies of midge damaged seed in resistant varietal blends and susceptible cultivars were similar. Consequently, *Sm1* did not protect against loss of market grade, but did increase market value due to larger yields especially when midge damage was high. Variation in damage and yield losses among the varietal blends suggested that wheat genotype influenced the expression of *Sm1*. The quantitative evaluation of resistance in these different backgrounds is leading to investigation of other genetic factors that may inhibit insect feeding that were not observed prior the incorporation of *Sm1* in spring wheat backgrounds.

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