



Genetic analysis of oviposition deterrence to orange wheat blossom midge in spring wheat

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Abstract

Key message A major QTL for oviposition deterrence to orange wheat blossom midge was detected on chromosome 1A in the Canadian breeding line BW278 that was inherited from the Chinese variety Sumai-3.

Abstract Orange wheat blossom midge (OWBM, *Sitodiplosis mosellana* Géhin, Diptera: Cecidomyiidae) is an important insect pest of wheat (*Triticum aestivum* L.) that reduces both grain yield and quality. Oviposition deterrence results in a reduction of eggs deposited on spikes relative to that observed on a wheat line preferred by OWBM. Quantification of oviposition deterrence is labor-intensive, so wheat breeders require efficient DNA markers for the selection of this trait. The objective of this study was to identify quantitative trait loci (QTL) for oviposition deterrence in a doubled haploid (DH) population developed from the spring wheat cross Superb/BW278. The DH population and check varieties were evaluated for OWBM kernel damage from five field nurseries over three growing seasons. QTL analysis identified major effect loci on chromosomes 1A (*QSm.mrc-1A*) and 5A (*QSm.mrc-5A*). Reduced kernel damage was contributed by BW278 at *QSm.mrc-1A* and Superb at *QSm.mrc-5A*. *QSm.mrc-1A* mapped to the approximate location of the oviposition deterrence QTL previously found in the American variety Reeder. However, haplotype analysis revealed that BW278 inherited this oviposition deterrence allele from the Chinese spring wheat variety Sumai-3. *QSm.mrc-5A* mapped to the location of awn inhibitor gene *BI*, suggesting that awns hinder OWBM oviposition. Single-nucleotide polymorphisms (SNPs) were identified for predicting the presence or absence of *QSm.mrc-1A* based upon haplotype. Functional annotation of candidate genes in 1A QTL intervals revealed eleven potential candidate genes, including a gene involved in terpenoid biosynthesis. SNPs for *QSm.mrc-1A* and fully awned spikes provide a basis for the selection of oviposition deterrence to OWBM.

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Abbreviations

ANOVA	Analysis of variance
BLAST	Basic local alignment search tool
BLUPs	Best linear unbiased predictors
DH	Doubled haploid
HMDK	Harvestable midge-damaged kernels
HD	Heading date
ICIM	Inclusive composite interval mapping
IM	Interval mapping
LOD	Logarithm of odds
MAS	Marker-assisted selection
MDK	Midge-damaged kernels
META-R	Multi Environment Trial Analysis with R
OWBM	Orange wheat blossom midge
HT	Plant height
PVE	Phenotypic variation explained
QTL	Quantitative trait loci
SNP	Single-nucleotide polymorphism
UMDK	Unharvestable midge-damaged kernels

Introduction

The orange wheat blossom midge (OWBM) (*Sitodiplosis mosellana* Géhin; Diptera: Cecidomyiidae) is among the most damaging insect pests of spring wheat, *Triticum aestivum* L., in western Canada, northern USA and many countries of Europe and Asia (Smith et al. 2014; Wise et al. 2015; Kassa et al. 2016). Recent outbreaks of OWBM have been reported in many wheat-growing European countries including UK, France, Belgium and Germany, causing significant yield losses (Thomas et al. 2005; Kassa et al. 2016). In China, two serious outbreaks caused by OWBM had been reported in the 1950s and 1980s, resulting in nearly 50% loss in wheat production (Zhang et al. 2020). In Canada, the first major outbreak of wheat midge occurred in northeast Saskatchewan in 1983 and caused over \$30 million in yield losses (Olfert et al. 2012). Since that time, OWBM has become a significant pest of spring wheat throughout all major wheat-growing areas of western Canada, causing seed damage, grade reduction and extensive yield losses during population outbreaks (Lamb et al. 1999; Olfert et al. 2009; Elliott et al. 2011). In Canada, the estimated annual wheat losses caused by OWBM exceeded \$60 million CAD, prior to the introduction of resistant wheat varieties carrying the OWBM resistance gene *Sm1* (Kassa et al. 2016).

The OWBM adults emerge from the soil at the time of wheat heading, mate and lay eggs (oviposit) on the emerging wheat spikes in response to characteristic wheat odor components (Barnes 1956). Upon hatching, larvae feed on developing kernels causing shriveled, cracked or distorted grains, contributing to poor seed quality and reduction in end-use suitability (Dexter et al. 1987; Lamb et al. 2000). Canadian spring and durum wheat varieties differ in their susceptibility to wheat midge damage. The severity of kernel damage by OWBM depends on several factors including larval population density (number of larvae present per spikelet), their spatial distribution and timing of oviposition relative to heading and anthesis (Wright and Doane 1987; Elliott and Mann 1996). Wheat spikes are most susceptible to damage when oviposition occurs during the heading stage and damage declines dramatically after the anthers are visible (Elliott and Mann 1996).

The development of wheat varieties with antibiotic resistance (antibiosis) and/or oviposition deterrence (antixenosis) to OWBM is an important component of integrated pest management (Lamb et al. 2002; Thomas et al. 2005). *Sm1* is the only described antibiosis gene for OWBM resistance (Berzonsky et al. 2002; McKenzie et al. 2002) and is located on wheat chromosome arm 2BS (Thomas et al. 2005; Kassa et al. 2016). The resistance mechanism of *Sm1* depends on antibiosis (inhibition

of larval growth) and mediated through the production of phenolic acids in the seed coat in response to larval feeding on the developing kernel. Phenolic acids such as ferulic acid and/or p-coumaric acid cause larvae to leave the kernel they are feeding on and die of starvation (Ding et al. 2000).

Oviposition deterrence is a resistance mechanism employed by plants to deter or reduce colonization by insects (Morando et al. 2015). Oviposition deterrence has been identified in both common and durum wheat (Lamb et al. 2001, 2002). Wheat varieties with oviposition deterrence to OWBM have fewer eggs laid on their spikes and are often associated with a higher proportion of eggs deposited on the rachis rather than the florets. The tiny, newly hatched larvae have to travel longer distances to feed on developing kernels and have a greater risk of desiccation en route (Lamb et al. 2002). Although the underlying mechanism that affects oviposition deterrence behavior in wheat is not fully understood, the deterrent wheat may have ability to repel females, provide an insufficient oviposition stimulus or physically block a female's access through chemical or morphological characteristics of wheat spikes during oviposition (Gharalari et al. 2009). Previous studies suggest that more than one gene controls oviposition deterrence to OWBM (Lamb et al. 2002; Gharalari et al. 2009). Blake et al. (2011) identified a major oviposition deterrence QTL on chromosome 1A (*QSm.mst-1A*) from spring wheat variety Reeder.

In this study, the genetic basis of oviposition deterrence to OWBM was explored using a DH population developed from a cross between Canadian hard red spring wheat variety Superb and oviposition deterrent breeding line BW278. The main objectives of this study were to: 1) identify QTL for oviposition deterrence and 2) identify SNP markers suitable for high-throughput marker-assisted selection (MAS).

Materials and methods

Plant materials and field trials

A DH population of 142 lines derived from the cross Superb/BW278 was used to identify QTL for oviposition deterrence to OWBM. Superb (pedigree: Grandin*2/AC Domain) is an awned, semi-dwarf spring wheat variety registered in the Canada Western Red Spring (CWRS) market class (Townley-Smith et al. 2010). BW278 (pedigree: AC Domain*2/Sumai-3) is a spring wheat breeding line with oviposition deterrence to OWBM that was developed to introgress *Fusarium* head blight (FHB) resistance from the Chinese variety Sumai-3 into Canadian germplasm. Sumai-3 is a well-known source of FHB resistance used in wheat breeding programs around the world (Dhokane et al. 2016). Field nurseries were grown from 2012 to 2014 at four locations

in western Canada (Winnipeg, Manitoba (Wpg); Glenlea, Manitoba (Gln); Brandon, Manitoba (Bdn); Saskatoon, Saskatchewan (Stn)). The spring wheat varieties Reeder, Waskada, 5602HR, Andrew, Harvest, Thatcher, Infinity, Lillian and AC Barrie were grown as control genotypes/checks in field tests. Field experiments were arranged in an alpha lattice design with 12 incomplete blocks and three replicates per environment. The experimental plot was a 10 cm row in which 15 seeds were sown. The Superb/BW278 population and checks were grown in field nurseries and exposed to natural populations of OWBM.

Phenotyping and statistical analysis

The Superb/BW278 DH population and checks were evaluated for OWBM kernel damage in five environments over three growing seasons (Wpg 2012, Gln 2013, Wpg 2013, Bdn 2014, Stn 2014). In this study, midge-damaged kernels (MDK) were quantified rather than counting eggs or larvae within spikes. Egg counting is extremely laborious and time-consuming, and must be done on fresh green spike samples, which was not possible based on the number of samples needed for QTL analysis. Counting larvae requires collection of green spikes and would also be time-consuming and difficult. Both methods are time-constrained as refrigerated material must be analyzed before eggs or larvae die. Therefore, MDK were used a proxy for egg counts and were quantified according to the protocol described previously (Wise et al. 2015). Briefly, wheat spikes were harvested at maturity from each plot, and 10 spikes per plot were examined for seed damage. Each spike was dissected with forceps under a dissection microscope and the number of damaged and total seeds found in each spike were recorded. MDK were separated by weight as being harvestable (> 8 mg; HMDK) or unharvestable (< 8 mg; UMDK) (Fig. 1). MDK, HMDK

and UMDK were expressed as a percentage of all kernels from the dissected spikes. Heading date (HD) and plant height (HT) data were also collected. HD was recorded as the Julian date at which 50% of spikes had emerged from flag leaf sheaths. HD data were collected in Winnipeg 2012, Brandon 2014 and Saskatoon 2014. HT was measured as the distance from the soil surface to the top of the wheat spikes (excluding awns). HT data were collected in Brandon and Saskatoon 2014. The presence and absence of awns on wheat spikes were also recorded.

The software Multi Environment Trial Analysis with R for Windows (META-R) Version 6.0 (Alvarado et al. 2017) was used for analysis of variance (ANOVA) and to calculate best linear unbiased predictors (BLUPs) for HD, HT, MDK, HMDK and UMDK in each individual environment and across all environments. Genotype (Gen), environment (Env), replicate (Rep) and incomplete block (Block) were considered random effects. Broad-sense heritability (H^2), least significant difference (LSD) and coefficient of variation (CV) were also calculated using META-R. META-R software uses the lme4 package in R software version 3.3.1. The linear model for lattice designs is:

$$Y_{ijkl} = \mu + \text{Env}_i + \text{Rep}_j(\text{Env}_i) + \text{Block}_k(\text{Env}_i, \text{Rep}_j) + \text{Gen}_l + \text{Env}_i \times \text{Gen}_l + \varepsilon_{ijkl}$$

where i is the i th environment, j is the j th replicate, k is the k th incomplete block and l is the l th genotype.

Genotyping

Genomic DNA was extracted from lyophilized leaf tissue with the DNeasy 96 Plant Kit (Qiagen, Toronto, Canada) and quantified using PicoGreen stain (Molecular Probes, Inc.,



Fig. 1 Orange wheat blossom midge (OWBM) symptoms caused by *Sitodiplosis mosellana* on common spring wheat: **a** sound, **b** harvestable midge-damaged kernels (HMDK) and **c** unharvestable midge-damaged kernels (UMDK)

Eugene, Oregon, USA). SNP markers were genotyped on the DH population and parents using the Illumina Infinium 90 K wheat SNP beadchip (Illumina, San Diego, CA) (Cavanagh et al. 2013; Wang et al. 2014). The raw data were analyzed with genotyping module of GenomeStudio version 2011.1 software (Illumina, San Diego, USA) using default clustering parameters.

Construction of genetic maps

A total of 142 DHs were used to construct a genetic linkage map of the Superb/BW278 population for mapping QTL for OWBM resistance and disease-related morphological traits. All monomorphic SNPs and those with > 10% missing data were excluded from linkage analysis. Each marker was tested for deviation from the expected 1:1 ratio using Chi-squared test. Markers showing significant ($P < 0.001$) segregation distortions were also discarded. Markers were placed into preliminary linkage bins using the BIN module in QTL Ici-Mapping version 4.0.6.0 (Li et al. 2007). A single marker with the least missing data was selected from each linkage bin and used for linkage analysis with MapDisto version 1.7.7 (Lorieux 2012). A minimum logarithm of the odds (LOD) score of 3.0 and maximum recombination fraction of 0.2 was used to identify linkage groups. Recombination fractions were converted into map distances using the Kosambi mapping function (Kosambi 1943). Linkage groups were assigned to chromosomes based on existing high-density consensus SNP maps of wheat (Maccaferri et al. 2014; Wang et al. 2014).

DNA markers were located on IWGSC (International Wheat Genome Sequencing Consortium) Chinese Spring RefSeq v1.0 (Appels et al. 2018) by Basic Local Alignment Search Tool (BLAST). The dwarfing gene *Rht-B1* (Peng et al. 1999), DNA markers for the earliness per se (*Eps*) QTL *QEet.fcu-5AL* (Liu et al. 2005) and the *B1* awn inhibitor (Huang et al. 2019) were also located on RefSeq version 1.0 with BLAST.

QTL analysis

QTL analysis was conducted with interval mapping (IM) and inclusive composite interval mapping (ICIM) using QTL Ici-Mapping version 4.1.0.0 (Li et al. 2007, 2008). For all traits, QTL analyses were carried out using BLUPs for test entries in each individual environment and pooled over all environments. Analysis for additive effect QTL was conducted with 0.1 cM steps, and the 5% LOD significance threshold was estimated with 10,000 permutations. The LOD significance threshold was 2.96 for both IM and ICIM additive effect QTL analysis. Additive effect QTL were declared when the LOD score exceeded the LOD significance threshold in two or more environments, or one or more environments plus

the pooled dataset, based upon IM or ICIM. QTL analysis statistics for these declared additive effect QTL were also reported for additional environments in which the LOD score exceeded 2.5. The proportion of phenotypic variance explained by each QTL was determined by the square of the partial correlation coefficient (R^2). Analysis for epistatic QTL was conducted with 2.0 cM steps and a default LOD significance threshold of 5.0. Epistatic QTL were reported when the LOD exceeded 5.0 in three or more environments, based upon QIME (i.e., interval mapping) and QICE (i.e., inclusive composite interval mapping) epistasis modules.

Haplotyping wheat lines with SNP markers linked to 1A QTL interval

Haplotype analysis was performed on a diverse collection of wheat germplasm, consisting of 75 wheat lines with varying levels of wheat midge resistance (Supplementary Table S6). Most wheat lines in the haplotype panel were susceptible to wheat midge (i.e., do not carry the OWBM resistance gene *Sm1*). The wheat lines BW278, Parshall, Reeder, Vesper and Waskada are known carriers of oviposition deterrence. A total of 159 Infinium SNP markers from the 1A QTL region were used for haplotype analysis to identify SNP markers for MAS.

Identification of the putative candidate genes (CGs) within the 1A QTL interval

SNP markers defining the boundaries of 1A QTL confidence interval were aligned to the IWGSC Chinese Spring RefSeq v1.0 (Appels et al. 2018) to define the physical location of the interval and identify putative CGs. Gene models found within the physical QTL intervals were retrieved using BioMart tool found in Ensembl Plants database (<https://plants.ensembl.org/index.html>). All genes in the candidate region were subjected to gene function annotation using GO analysis, and biological functions of a gene product were described with GO Term. GO annotations can be found in the IWGSC database (Appels et al. 2018). The metabolic pathway annotation was carried out using Blast2GO (<https://www.blast2go.com/>) against KEGG database (<https://www.genome.jp/kegg/pathway.html>) for genes in the 1A QTL interval.

Results

Phenotypic data analyses

Histograms of MDK, HD and HT in Superb/BW278 DH population are illustrated in Fig. 2. BW278 and the oviposition deterrent check lines Reeder and Waskada had fewer

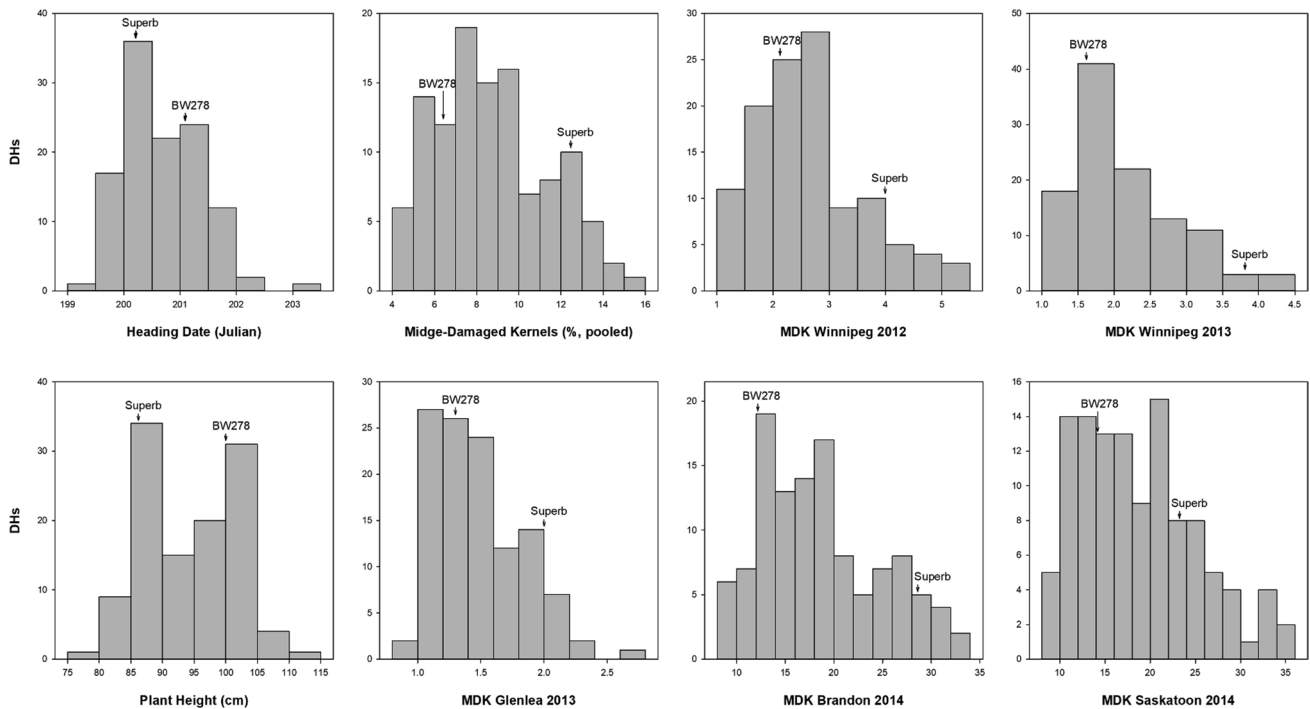


Fig. 2 Frequency distribution of best linear unbiased predictors (BLUPs) for orange wheat blossom midge (OWBM, *Stodiplosis mosellana*)-related phenotypic traits: heading date, plant height and

midge-damaged kernel (MDK) in the Superb/BW278 DH population in five environments over 2012, 2013 and 2014. Means of the parents are indicated

MDK relative to the mean of the Superb/BW278 DH population (Table 1). Superb, Andrew, Harvest, Thatcher, Infinity, Lillian, AC Barrie and Intrepid were more susceptible to OWBM than mean of the Superb/BW278 DH population. Superb flowered almost a day earlier and was 14 cm shorter than BW278. The average plant height among the 142 DHs was 94 cm, and average heading date was Julian date 201. There was some transgressive segregation for plant height, but most DHs were within the means of the parents. Heading date was approximately normally distributed in the Superb/BW278 populations (Fig. 2). Analysis of variance showed significant ($P < 0.001$) differences among genotypes for all traits (HD, HT, MDK, HMDK and UMDK) (Table 2 and Supplementary Table S1). Broad-sense heritability estimates were 0.75 for heading date, 0.88 for plant height, 0.73 for MDK, 0.74 for HMDK and 0.69 for UMDK in the Superb/BW278 DH population (Table 2). Broad-sense heritability estimates for individual environments are presented in Supplementary Table S2. For each individual trait, the data were positively correlated in all pairwise comparison between the environments (Supplementary Table S3). Correlation analysis revealed a significant positive correlation between midge damage (MDK, HMDK, UMDK) and HD,

and a negative correlation between midge damage (MDK, HMDK, UMDK) and HT (Table 3).

Linkage map

A high-density whole genome linkage map was developed for the Superb/BW278 DH population using the 90 K wheat Infinium SNP chip. The Superb/BW278 linkage map consisted of 7,158 SNPs. The total map length across the 32 linkage groups was 1882.3 cM (Supplementary Table S4). In some instances, a chromosome consisted of two or three linkage groups (e.g., chromosomes 2A, 2D, 3B, 4D, 5A, 5B, 6A, 6D and 7D), which was not unexpected since some regions of the genome will be identical by descent given the relatedness of the parents. Of the 7,158 polymorphic markers, 2,803 SNPs (39.2%) mapped to the A genome spanning 618 cM, 3,993 SNPs (55.8%) mapped to the B genome covering 619 cM, and 362 SNPs (5.1%) mapped to the D genome spanning 645 cM. The average spacing between neighboring markers was 0.3 cM (Supplementary Table S5). The *B1* awn inhibitor gene mapped to chromosome arm 5AL (linkage group 5A.2 at position 27.1 cM) (Supplementary Table S4).

Table 1 Best linear unbiased predictors (BLUPs) of checks and descriptive statistics of the Superb/BW278 DH population for heading date (HD), plant height (HT) and midge-damaged kernels (MDK) in field nurseries and pooled over environments

Line	HD ^a	HT ^b	MDK ^c					
			Pooled	Wpg12 ^d	Gln13	Wpg13	Bdn14	Stn14
<i>Superb/BW278 DH population</i>								
Mean	200.7	94.1	8.7	2.7	1.5	2.1	18.7	18.7
Min ^e	199.2	78.2	4.4	1.2	0.9	1.0	8.3	8.7
Max ^f	203.2	114.2	15.1	5.4	2.8	4.3	33.5	35.9
Std dev ^g	0.7	7.4	2.6	1.0	0.3	0.8	6.1	6.6
<i>Parents</i>								
Superb	200.2	86.2	12.5	4.0	2.0	3.8	28.6	23.2
BW278	201.1	100.0	6.4	2.1	1.3	1.6	12.1	14.1
<i>Checks</i>								
Reeder	200.3	81.5	6.6	1.8	1.5	1.8	14.5	13.3
Waskada	201.2	96.4	7.0	1.6	1.3	1.9	12.4	18.0
5602HR	200.6	93.0	8.7	2.0	1.8	2.9	19.8	16.9
Andrew	202.4	80.6	10.3	3.8	2.2	2.6	18.8	23.4
Harvest	200.6	86.6	10.8	2.8	2.2	3.0	20.9	24.8
Thatcher	201.0	101.3	11.0	5.9	1.3	1.6	20.1	26.6
Infinity	203.1	92.1	11.7	4.6	1.8	4.3	15.4	31.6
Lillian	201.8	97.1	12.1	3.8	2.6	2.4	22.8	28.1
AC Barrie	201.6	99.2	12.6	5.3	1.5	2.9	26.8	26.7
Intrepid	199.9	88.0	16.0	4.2	3.1	4.8	35.2	31.6

^aJulian date

^bCentimetres

^cPercentage

^dWpg, Winnipeg, MB; Gln, Glenlea, MB; Bdn, Brandon, MB; Stn, Saskatoon, SK; 12, 2012; 13, 2013; 14, 2014

^eMinimum

^fMaximum

^gStandard deviation

Table 2 Estimated variance components for heading date (HD), plant height (HT), midge-damaged kernels (MDK), harvestable midge-damaged kernels (HMDK) and unharvestable midge-damaged kernels (UMDK) in the Superb/BW278 DH population grown in Winnipeg 2012, Glenlea 2013, Winnipeg 2013, Brandon 2014 and Saskatoon 2014

Source of Variation	HD		HT		MDK		HMDK		UMDK	
Genotype ($\hat{\sigma}_G^2$)	0.74	***	63.48	***	10.30	***	1.28	***	5.10	***
Environment ($\hat{\sigma}_E^2$)	237.09	***	13.72	ns	83.36	***	7.26	***	41.99	***
G x E ^a ($\hat{\sigma}_{GE}^2$)	0.28	***	0.00	ns	12.60	***	1.32	***	7.35	***
Residual ($\hat{\sigma}^2$)	1.37		54.07		19.28		2.68		11.98	
Grand mean	200.71		93.89		8.83		2.96		5.87	
LSD ^b	1.23		8.38		4.81		1.66		3.61	
CV (%) ^c	0.58		7.83		49.74		55.30		58.96	
Heritability	0.75		0.88		0.73		0.74		0.69	

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns $P \geq 0.05$

^aGenotype x environment

^bLeast significant difference ($P = 0.05$)

^cCoefficient of variation

Heading date QTL

A major QTL for heading date was identified on the chromosome 5A in the Superb/BW278 DH population

(linkage group 5A.2 at approximately 12 cM) (Table 4). Superb carried the early allele at *QHD.mrc-5A*. These results were consistent with Superb being 0.9 day earlier heading than BW278. *QHD.mrc-5A* mapped to the

Table 3 Phenotypic correlations (r) among OWBM-related traits (heading date, plant height and midge-damaged kernels) assessed in the Superb/BW278 DH population and corresponding statistical significance

Traits ^a	HD		MDK		UMDK		HMDK	
HT	- 0.11	ns	- 0.31	***	- 0.30	***	- 0.25	**
HD			0.24	**	0.20	*	0.27	**
MDK					0.97	***	0.85	***
UMDK							0.70	***

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns $P \geq 0.05$

^aHD, heading date; HT, plant height; MDK, midge-damaged kernels; HMDK, harvestable midge-damaged kernels; UMDK, unharvestable midge-damaged kernels

expected location of earliness per se (*Eps*) QTL *QEet.fcu-5AL* from American hard red spring wheat variety Grandin (Liu et al. 2005).

Plant height QTL

A major additive effect QTL for plant height was identified on chromosome 4B at approximately 32 cM and was named *QHt.mrc-4B* (Table 4). The BW278 allele increased plant height at this QTL, consistent with BW278 being 14 cm taller than Superb in the field experiments. *QHt.mrc-4B* explained 58.3% to 73.8% of the phenotypic variation and is consistent with the location of *Rht-B1* locus.

Oviposition deterrence QTL

Six additive effect QTL for oviposition deterrence (MDK, HMDK and UMDK) were identified using IM and ICIM analysis with the additive effect module of QTL IciMapping (Table 4). BW278 contributed midge resistance at four of these QTL, which is consistent with BW278 showing fewer MDK than Superb (Table 1 and unpublished data, Marjorie Smith). BW278 contributed resistance at *QSm.mrc-1A* (chromosome 1A at 105.4 cM), which was consistent with oviposition deterrence QTL *QSm.mst-1A* reported previously from the American oviposition deterrent spring wheat variety Reeder (Blake et al. 2011). The *QSm.mrc-1A* was detected under all five environments with the LOD scores ranging from 4.6 to 31.6 and explained 10.9% to 58.3% of the phenotypic variation (Fig. 3 and Table 4). A second major oviposition deterrence QTL *QSm.mrc-4B* mapped on chromosome 4B (31.9 cM) coincided with HT QTL *QHt.mrc-4B*. At the plant height QTL *QHt.mrc-4B* (i.e., *Rht-B1*), the reduced height allele was consistently associated with increased OWBM damage.

The most prominent oviposition deterrence QTL from Superb mapped on chromosome 5A (*QSm.mrc-5A*), explaining up to 21% of the phenotypic variation. This 5A QTL was statistically significant in three of the five individual environments and the pooled dataset. *QSm.mrc-5A* mapped

approximately 15 cM distal of the HD QTL *QHd.mrc-5A* (Table 4 and Supplementary Table S4), which explains the positive correlation between HD and OWBM damage in the Superb/BW278 population. *QSm.mrc-5A* coincided with the *B1* awn inhibitor gene (Huang et al. 2019), which mapped to chromosome arm 5AL (linkage group 5A.2) at position 27.1 cM (Table 4 and Supplementary Table S4). Figure 4 illustrates the combined effects of the two major QTL *QSm.mrc-1A* and *QSm.mrc-5A* on MDK.

Three minor QTL for oviposition deterrence were identified on chromosomes 3B and 7A (*QSm.mrc-3B*, *QSm.mrc-7A.1* and *QSm.mrc-7A.2*) based on ICIM, which were not detected by IM. Their effects were lower and less consistent than the QTL on chromosomes 1A, 4B and 5A (Table 4). In addition, these QTL were detected based upon HMDK or UMDK data, but not detected based upon total MDK.

Epistatic QTL were not detected for oviposition deterrence or other phenotypic traits (heading date and plant height) with the QIME (i.e., interval mapping) and QICE epistatic module (i.e., inclusive composite interval mapping) at the LOD significance threshold of 5.

Haplotype analysis

Haplotype analysis of a panel of wheat lines revealed that the *QSm.mrc-1A* region had considerable haplotype diversity among the spring wheat varieties and breeding lines. The parent BW278 (pedigree: AC Domain*2/Sumai-3) had the same haplotype as Chinese wheat variety Sumai-3, but not AC Domain, which indicates that BW278 inherited *QSm.mrc-1A* from Sumai-3 (BW278 haplotype). The Canada Western Red Spring (CWRS) variety Waskada which has a pedigree of BW278/2*Superb inherited this chromosome region from BW278 (Supplementary Table S6). Interestingly, BW278 had a different haplotype at *QSm.mrc-1A* than the American spring wheat variety Reeder, carrier of the *QSm.mst-1A* allele located at the same genomic region (Blake et al. 2011). Known OWBM oviposition deterrent varieties Reeder and Parshall had similar but slightly different haplotypes at *QSm.mrc-1A*. Vesper, another source of

Table 4 Additive effect QTL identified for OWBM (*Sitodiplosis mosellana*)-related traits (heading date, plant height and midge-damaged kernels: MDK, HMDK and UMDK) in the Superb/BW278 DH population

QTL designation	Trait—environment combinations ^c	LG ^d	IM ^a				ICIM ^b				
			Pos ^e	LOD ^f	PVE ^g	Add ^h	Pos	LOD	PVE	Add	
Heading date (HD)											
<i>QHd.mrc-5A</i>	HD_avg	5A.2	13.5	6.61	23.2	-0.34	13.5	6.61	23.2	-0.34	
	HD_Wpg12	5A.2	13.6	6.85	24.1	-0.48	13.6	10.31	15.9	-0.45	
	HD_Stn14	5A.2	10.0	5.06	18.3	-0.32	10.0	5.06	18.3	-0.32	
Height (Ht)											
<i>QHt.mrc-4B</i>	Ht_avg	4B	31.9	33.45	73.8	-6.33	31.9	33.45	73.8	-6.33	
	Ht_Bdn14	4B	31.9	29.48	58.3	-6.48	31.9	29.48	69.3	-6.48	
	Ht_Stn14	4B	31.9	23.06	60.3	-4.61	31.9	23.06	60.3	-4.61	
Midge-damaged kernels (MDK)											
<i>QSm.mrc-1A</i>	MDK_avg	1A	104.7	18.77	57.7	1.89	105.4	31.60	55.3	2.00	
	MDK_Wpg12	1A	105.4	10.80	35.3	0.56	105.4	14.68	36.8	0.59	
	MDK_Gln13	1A	105.4	6.82	24.0	0.17	105.4	6.82	24.0	0.17	
	MDK_Wpg13	1A	102.6	7.91	24.5	0.40	102.6	8.80	28.1	0.40	
	MDK_Bdn14	1A	105.4	20.46	56.0	4.58	105.4	28.40	58.3	4.88	
	MDK_Stn14	1A	104.0	9.99	36.3	3.77	104.4	23.75	34.3	4.25	
	HMDK_avg	1A	104.2	15.56	51.1	0.64	104.0	29.10	45.2	0.65	
	HMDK_Wpg12	1A	105.4	7.83	27.0	0.21	105.4	9.10	7.9	0.20	
	HMDK_Gln13	1A					105.4	5.82	0.8	0.06	
	HMDK_Wpg13	1A	102.6	4.64	10.9	0.12	102.6	6.68	19.1	0.14	
	HMDK_Bdn14	1A	103.3	13.24	41.7	1.06	104.3	18.13	41.6	1.10	
	HMDK_Stn14	1A	104.2	10.22	37.2	1.44	103.3	22.37	37.8	1.56	
	UMDK_avg	1A	105.0	15.44	46.4	1.19	105.4	29.00	15.2	1.23	
	UMDK_Wpg12	1A	105.1	9.76	32.7	0.29	106.1	13.35	34.0	0.30	
	UMDK_Gln13	1A	105.4	4.75	17.5	0.07	105.4	4.75	17.5	0.07	
	UMDK_Wpg13	1A	102.6	7.06	25.4	0.26	102.6	7.06	25.4	0.26	
	UMDK_Bdn14	1A	105.4	18.11	51.6	3.34	105.4	25.85	52.1	3.53	
	UMDK_Stn14	1A	103.8	7.06	27.0	2.24	104.4	14.37	4.9	2.30	
	<i>QSm.mrc-3B</i>	HMDK_avg	3B.2					12.0	6.28	5.9	0.24
		HMDK_Bdn14	3B.2					12.6	3.17	5.3	0.39
HMDK_Stn14		3B.2					2.1	3.10	3.4	0.47	
<i>QSm.mrc-4B</i>	MDK_avg	4B					33.4	8.59	8.9	0.80	
	MDK_Wpg12	4B					33.4	3.34	6.6	0.25	
	MDK_Wpg13	4B	48.5	3.22	12.1	0.29	33.3	3.27	9.3	0.23	
	MDK_Bdn14	4B					31.9	5.15	6.3	1.61	
	MDK_Stn14	4B					31.9	11.99	14.1	2.73	
	UMDK_avg	4B					32.7	11.37	4.0	0.63	
	UMDK_Bdn14	4B					31.9	6.12	8.0	1.38	
<i>QSm.mrc-5A</i>	UMDK_Stn14	4B					33.4	6.29	1.8	1.42	
	MDK_avg	5A.2	27.1	2.52	10.4	-0.81	27.8	11.74	12.9	-0.97	
	MDK_Wpg12	5A.2					27.2	3.83	7.6	-0.27	
	MDK_Bdn14	5A.2					27.8	2.66	3.1	-1.13	
	MDK_Stn14	5A.2	27.1	5.41	21.2	-2.90	27.8	14.07	17.3	-3.03	
	HMDK_avg	5A.2	27.1	2.64	11.1	-0.30	27.1	15.23	17.2	-0.41	
	HMDK_Bdn14	5A.2					27.2	4.60	7.9	-0.48	
	HMDK_Stn14	5A.2	27.1	3.11	12.9	-0.85	27.8	12.34	16.3	-1.03	
	UMDK_avg	5A.2					27.8	7.99	2.6	-0.51	
	UMDK_Wpg12	5A.2					27.8	4.06	8.5	-0.15	
UMDK_Stn14	5A.2	27.1	5.25	20.6	-1.97	27.1	9.59	3.0	-1.82		

Table 4 (continued)

QTL designation	Trait—environment combinations ^c	LG ^d	IM ^a				ICIM ^b			
			Pos ^e	LOD ^f	PVE ^g	Add ^h	Pos	LOD	PVE	Add
<i>QSm.mrc-7A.1</i>	UMDK_avg	7A					113.7	4.30	1.4	-0.38
	UMDK_Bdn14	7A					112.8	5.46	7.1	-1.31
<i>QSm.mrc-7A.2</i>	HMDK_avg	7A					164.9	5.60	5.2	0.22
	HMDK_Bdn14	7A					164.9	2.69	4.5	0.36
	HMDK_Stn14	7A					164.8	5.07	5.9	0.62

^aIM, interval mapping

^bICIM, inclusive composite interval mapping

^cHD, heading date; HT, plant height; MDK, midge-damaged kernels; HMDK, harvestable midge-damaged kernels; UMDK, unharvestable midge-damaged kernels; Wpg12, Winnipeg 2012; Gln13, Glenlea 2013; Wpg13, Winnipeg 2013; Bdn14, Brandon 2014; Stn14, Saskatoon 2014

^dLG, linkage group

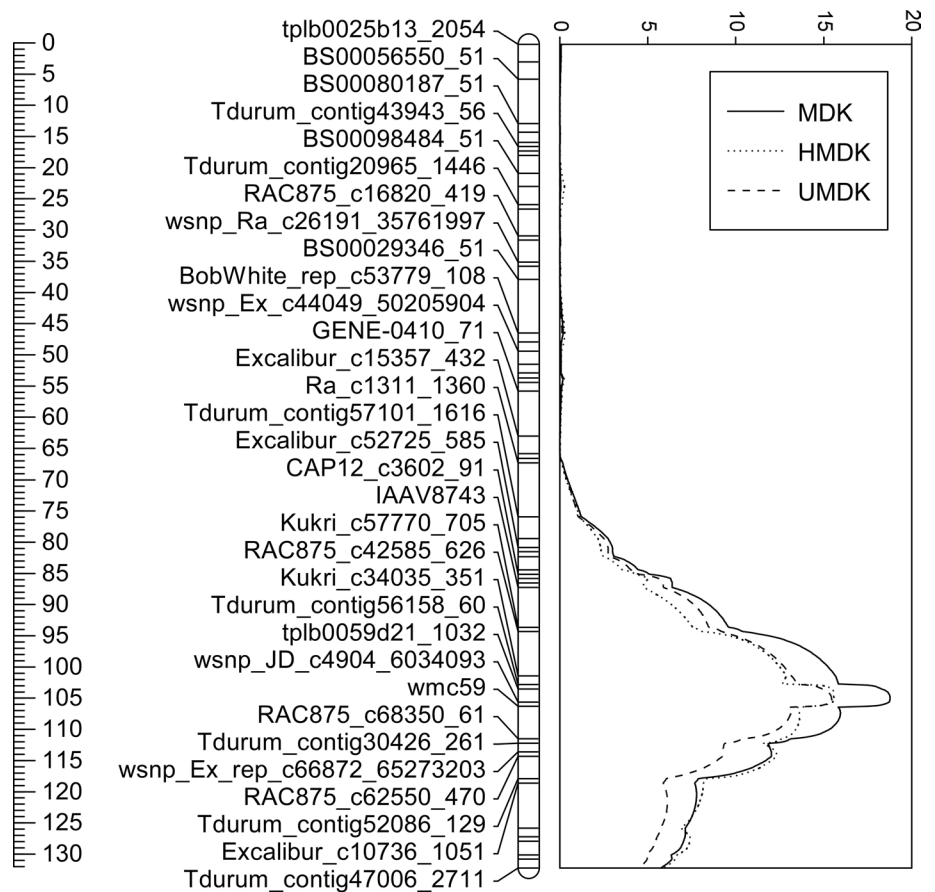
^ePos, position (cM)

^fLOD, peak LOD score; LOD significance threshold = 2.96 (IM and ICIM)

^gPVE, phenotypic variation explained (r^2 ; %)

^hAdd, additive effect of allele substitution. A positive number indicated that the ‘Superb’ allele increased the respective quantitative trait, and vice versa

Fig. 3 QTL *QSm.mrc-1A* (interval mapping) on chromosome 1A confers oviposition deterrence to orange wheat blossom midge (OWBM, *Sitodiplosis mosellana*) in common spring wheat. The ruler is scaled in centiMorgans (cM). The LOD threshold value for declaring QTL was 2.96



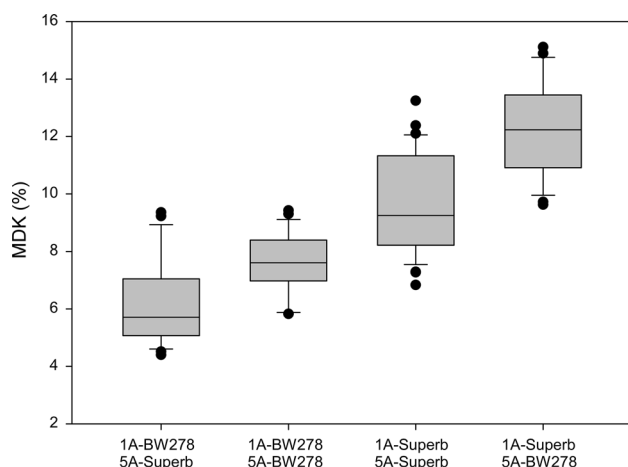


Fig. 4 Box plots of midge-damaged kernel (MDK) data for the four genotypic classes defined by the two major oviposition deterrence QTL *QSm.mrc-1A* and *QSm.mrc-5A* with the loci *wspn_JD_c4904_6034093* and *B1*, respectively

oviposition deterrence had the same haplotype as OWBM susceptible wheat Roblin (Roblin haplotype). Spring wheat AC Splendor had the same haplotype as the susceptible parent Superb (Superb haplotype), suggesting it also lacks the oviposition QTL *QSm.mrc-1A*. A majority of wheat lines in the diversity wheat panel did not have the haplotype of either BW278 or Superb (Supplementary Table S6).

Accurate MAS of *QSm.mrc-1A* can be accomplished with combinations of the following SNPs: *wspn_Ex_c28900_37982485*, *BS00023935_51*, *RAC875_c41993_582*, *wspn_CAP8_c4785_2322876*, *wspn_BE443588A-Ta_2_2*, *TA005289-1104*, *BS00070560_51*, *Excalibur_c23598_1632*, *BobWhite_c44164_151*, *Tdurum_contig81011_244*, *wspn_CAP11_c146_160903*, *CAP12_c6629_301* and *Tdurum_contig62584_770*. These SNPs differentiate the BW278 haplotype from other haplotypes (Supplementary Table S6). The spring wheat varieties Frontana, Nyubai and Wangshuibai had similar haplotypes to BW278 from 94.1 to 103.3 cM, but differed from 105.4 to 111.3 cM (Supplementary Table S6). Different combinations of SNPs may be needed depending on the germplasm present in specific breeding programs. Presently, BW278 and Reeder carry the only two haplotypes known to confer OWBM oviposition deterrence on chromosome 1A.

Identification of putative candidate genes (CGs) at *QSm.mrc-1A*

QSm.mrc-1A mapped to a 3.6 cM interval (102.5–106.1 cM) on chromosome 1A linkage map which corresponded to a 42.7 Mb (536,613,453–579,299,659 bp) physical region in the IWGSC Chinese Spring reference genome RefSeq v1.0 (Appels et al. 2018) based upon BLAST locations

of SNP markers defining the boundaries of *QSm.mrc-1A*. The 42.6 Mb candidate region on chromosome 1A contained 815 protein coding genes. Based on gene function annotation, 11 CGs may be related to OWBM resistance (Supplementary Table S7). In the candidate region, three genes (*TraesCS1A02G355300*, *TraesCS1A02G398100* and *TraesCS1A02G398200*) had predicted biological function in defense responses. The CG *TraesCS1A02G384300* was predicted to be involved in the biosynthesis of terpenoids. The gene *TraesCS1A02G382900* was annotated as a serine/threonine protein phosphatase 2A regulatory subunit, and genes *TraesCS1A02G382900* and *TraesCS1A02G389500* were involved in signal transduction pathways. Several other annotated genes carrying domains that may have roles in plant defense against insects such as hydrolases superfamily protein and protein kinase were also identified (Supplementary Table S7).

Discussion

OWBM-resistant wheat varieties carrying the antibiosis gene *Sm1* have been successfully used to manage OWBM in Canada, USA and Europe (<https://midgetolerantwheat.ca/>; <https://ahdb.org.uk/wheat-blossom-midges>; <https://www.usda.gov/>). However, the heavy reliance on *Sm1* leaves wheat crops vulnerable if *Sm1*-virulent OWBM populations evolve. Therefore, alternate genetic resources to control OWBM are needed. To date, very few alternate genetic resistances to *Sm1* have been identified. For example, Blake et al. (2011) identified a QTL (*QSm.mst-1A*) that reduces midge damage in the American Spring wheat variety Reeder and Zhang et al. (2020) detected two QTL on chromosome 4A conferring resistance to OWBM from Chinese wheats Henong215 and Jimai24.

In this study, the genetic basis of oviposition deterrence was characterized in a DH population derived from the cross between a Canadian spring wheat variety Superb and breeding line BW278. QTL analysis identified six QTL controlling oviposition deterrence in the Superb/BW278 population on chromosomes 1A, 3B, 4B, 5A and 7A, which are different from either the location of *Sm1* (Thomas et al. 2005) or two recently reported resistance QTL on chromosome 4A (Zhang et al. 2020). BW278 contributed resistant alleles at four these loci, which is consistent with BW278 being more deterrent to oviposition in the field nurseries (Table 1). A major QTL *QSm.mrc-1A*, on chromosome 1A that was associated with reduced midge-damaged kernels was identified in BW278. This QTL mapped to the same region of chromosome 1A as the previously reported oviposition deterrence QTL *QSm.mst-1A* in the American spring wheat variety Reeder (Blake et al. 2011). However, haplotype analysis using 159 Infinium

SNP markers in this region of chromosome 1A in a panel of wheat lines revealed that BW278 (AC Domain*2/Sumai-3) inherited this region from the Chinese spring wheat Sumai-3, which has been used as a major source of FHB resistance in Canadian spring wheat breeding programs (Dhokane et al. 2016). The OWBM oviposition deterrent variety Waskada (BW278/2*Superb) (Fox et al. 2009) had the same haplotype as BW278, as did the Canadian soft white spring variety Sadash. Interestingly, BW278 had a different haplotype in the 1A QTL region than the oviposition deterrent variety Reeder, carrier of *QSm.mst-1A* (Blake et al. 2011).

Functional annotation of genes within the *QSm.mrc-1A* confidence interval of 42.7 Mb (536,613,453–579,299,659 bp) revealed 11 OWBM resistance-related candidate genes (CGs). Several different classes of insect resistance genes have been reported in plants, including genes encoding inhibitors of proteases (serine and cysteine) and alpha-amylase, plant lectins and enzymes such as chitinases and lipoxygenases (Gatehouse and Gatehouse 1998; Malone et al. 2008). However, few genes have been implicated in OWBM resistance (Hao et al. 2019). TraesCS1A02G384300 has been annotated as a gene involved in chemical reactions and pathways resulting in the biosynthesis of terpenoids. As reported previously, female midge uses fine-scale features and surface chemicals, including volatiles, to select oviposition sites on the wheat spike (Gharalari et al. 2009, 2012). Volatile organic compound(s) produced by deterrent genotypes may reduce oviposition on preferred genotypes and may cause the females to lay their eggs further from potential larval feeding sites (Lamb et al. 2003; Gharalari et al. 2009). No morphological trait has been identified that accounts for oviposition preferences, apart from a small effect of inter-spikelet distance (Lamb et al. 2001; Gharalari et al. 2009).

The oviposition deterrence QTL on chromosome 4B from BW278 is collocated with a major gene affecting plant height in the Superb/BW278 DH population. There was a strong association between the dwarfing alleles at *Rht-B1* and increased kernel damage (*QSm.mrc-4B*) in the Superb/BW278 population (Table 4). Superb has the dwarfing allele *Rht-B1b*, which was associated with increased kernel damage at *QSm.mrc-4B*. Despite the widespread use of *Rht-B1b* and *Rht-D1b* in modern wheat varieties for increasing wheat grain yields and providing lodging resistance, their associations with increased susceptibility to some plant pathogens have been reported several times in past studies (Srinivasachary et al. 2009; Saville et al. 2011). Previously, a QTL mapping study of yield and yield components in a spring wheat cross between a high-yielding variety, Superb (Grandin*2/AC Domain) and BW278 revealed that Superb carried a dwarfing allele at *Rht-B1* from Grandin and associated with increased yield (<https://mspace.lib.umanitoba.ca/>

[bitstream/handle/1993/21231/Cuthbert_Molecular_Mapping.pdf](https://mspace.lib.umanitoba.ca/bitstream/handle/1993/21231/Cuthbert_Molecular_Mapping.pdf)), but also provides a microclimate more favorable for pathogen establishment due to reduced height (Scott et al. 1985). However, this association between the *Rht-B1b* and increased OWBM damage may not translate into increased OWBM damage in commercial fields. OWBM may preferentially oviposit on short genotypes in small plot experiments, possibly to avoid the wind. This would explain the correlation observed between plant height and OWBM damage in this study. Such an effect may not occur in commercial fields planted to monoculture.

The awned parent Superb contributed oviposition deterrence at the QTL *QSm.mrc-5A*, which collocated with the position of the *B1* awn inhibitor locus (Huang et al. 2019) in the Superb/BW278 population. *QSm.mrc-5A* and *B1* mapped approximately 15 cM distal of the HD QTL *QHd.mrc-5A* on chromosome 5A (Table 4 and Supplementary Table S4). Since *QHd.mrc-5A* does not collocate with *QSm.mrc-5A*, the significant correlation between HD and OWBM kernel damage in the Superb/BW278 DH population is most likely due to linkage rather than pleiotropy (Tables 3, 4). The earlier maturing parent Superb contributed earliness allele at the HD QTL *QHd.mrc-5A* in the Superb/BW278 DH population. Earliness per se genes (*Eps*) are known to regulate flowering time independently of vernalization genes (*Vrn*) and photoperiod genes (*Ppd*), and are important for the fine-tuning of flowering time (Lewis et al. 2008). *Eps* genes have been reported to induce earlier flowering, even in the presence of *Vrn* and *Ppd* genes (van Beem et al. 2005), and they have been mapped as QTL for heading time on different chromosomes on wheat (Kamran et al. 2013; Zanke et al. 2014). Liu et al. (2005) identified a major *Eps* QTL designated as *QEet.fcu-5AL* on chromosome 5A for earliness contributed by Grandin in the Grandin/BR34 RIL population. Grandin is a parent of Superb (pedigree: Grandin*2/AC Domain).

Additional QTL for oviposition deterrence were identified on chromosomes 3B and 7A, and their effects were lower and less consistent than the major oviposition deterrence QTL on chromosomes 1A, 4B and 5A. Two minor effect QTL, *QSm.mrc-3B* and *QSm.mrc-7A.2* detected based on HMDK from BW278, and one minor QTL, *QSm.mrc-7A.1* detected based on UMDK from Superb, were also responsible for reducing OWBM damage in the Superb/BW278 DH population (Table 4).

Gharalari et al. (2009) suggested that oviposition deterrence is controlled by multiple genes, with complementary interaction among genes. Difficult phenotyping and complex genetics would make it difficult to incorporate this trait into breeding programs. The present genetic study revealed that oviposition deterrence was controlled by several genes. However, *QSm.mrc-1A* had a major impact on MDK such that MAS of this QTL combined with

phenotypic selection of awned genotypes would provide a basis for efficient selection of oviposition deterrence to OWBM. The 11 OWBM candidate genes for *QSm.mrc-1A* reported in this study would be an appropriate set of genes for follow-up genetic research on oviposition deterrence. In conclusion, the findings of this study provide insight into the inheritance of oviposition deterrence to OWBM and provide information for pyramiding the OWBM resistance gene *Sm1* with oviposition deterrence.

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Author contribution statement All authors planned and organized the study. CM, IW, MS and SF designed the study. CM, CP, SK and AB grew field nurseries. CM, IW, MS and AC assessed OWBM damage. DT and CM developed the linkage maps, conducted QTL analyses, interpreted results and wrote the first draft of the manuscript. All authors contributed to and approved the final manuscript.

Availability of data and material Data supporting the current study can be obtained by contacting the corresponding author (curt.mccartney@canada.ca).

Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and animals participants This article does not contain any studies with human participants or animals performed by any of the authors.

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