OVIPOSITION BEHAVIOR OF WHEAT MIDGE *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) AND INHERITANCE OF DETERRENCE RESISTANCE IN SPRING WHEAT

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Dedicated to

the best teacher I have ever had,

Marjorie Ann Henderson Smith

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Ali Hosseini Gharalari; Ph.D., University of Manitoba, 2008.

Oviposition behavior of wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), and inheritance of deterrence resistance in spring wheat

Co-advisors: Dr. M.A.H. Smith & Dr. S.L. Fox

Wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), is a key pest of wheat, *Triticum aestivum* L. (Poaceae), in the Canadian Prairies. The larvae destroy wheat kernels, resulting in reduction of quality and quantity of wheat. Deployment of antixenotic wheat lines, which suppress oviposition of wheat midge, can reduce damage in wheat fields. The objectives of this thesis were to explore the interactions between wheat midge and spring wheat with emphasis on oviposition behavior and to explore the antixenosis of wheat to oviposition from the point of view of genetics and crop breeding. In this research, a doubled-haploid spring wheat population was studied, which was the progeny of a cross between a susceptible wheat cultivar 'Roblin' and a resistant (antixenotic and antibiotic) wheat line 'Key 10'. Oviposition of wheat midge on wheat spikes in the laboratory was affected by visual and chemical cues. The visual contrast between wheat spikes and the background color in the laboratory was important in modifying oviposition of wheat midge on wheat spikes. Low contrast resulted in low egg density on wheat spikes in the laboratory. The egg density on wheat

spikes in the laboratory decreased when the background color of the spikes was red or black; while yellow and blue backgrounds did not decrease egg density on the spikes. The laboratory study provided evidence that wheat midge oviposition was affected by volatiles emitted by wheat spikes. The volatiles of spikes of a post-anthesis susceptible wheat cultivar, 'Roblin', and a pre-anthesis resistant wheat line, 'Key 10', significantly suppressed the oviposition of wheat midge in the laboratory. It is hypothesized that these volatiles might be a factor in antixenosis of wheat against wheat midge in the doubled-haploid population studied. It is suggested that the differences of oviposition behavior in susceptible and antixenotic wheats, which was observed in the laboratory, might be due to volatiles emitted by wheat spikes. However, other factors such as tactile cues might also be involved. The observation of oviposition behavior in the laboratory on the susceptible wheat cultivar 'Roblin' showed that wheat midge started ovipositing sooner, stayed longer, laid more eggs and left the spike sooner after the last oviposition than on the antixenotic line 'Key 10'. However, the time required for laying one egg was similar when wheat midge was on the susceptible or resistant wheat. The observed antennation behavior of wheat midge while probing the wheat spike might indicate that wheat midge probed for chemical cues emitted by the host plant. The observed ovipositor tapping and dragging on the wheat spike surface while probing the spike suggested that there might be receptors at the tip of the ovipositor which receive tactile cues from the plant surface, guiding oviposition. The correlations between morphological traits of bread wheat spikes and antixenosis in the laboratory were not high enough to conclude that those traits were associated with antixenosis. However, more research on fine scale morphological traits of the spike may reveal relationships with antixenosis. Based on data from a laboratory trial

and trials in the field over two field seasons, it was concluded that the antixenosis to wheat midge in the doubled-haploid population was probably conferred by two genes with complementary interactions among genes, and a heritability of 67%. In the two field seasons, the least preferred line received 13% and 11% as many eggs as on 'Roblin'; 'Key 10' received 57% and 20% as may eggs as on 'Roblin'. Our study did not provide evidence for linkage between antixenosis genes and the antibiosis gene, *Sm1,* which is associated with death of larvae of wheat midge. The antixenosis of spring wheat against wheat midge can be considered as a promising mechanism for suppressing wheat midge oviposition in the field. More research is required to reveal additional genetic information which would help crop breeders in production of cultivars antixenotic to wheat midge.

Introduction

The wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), is a key pest of wheat in the northern Great Plains (Wright and Doane 1987; Lamb *et al.* 2000*a*). Larvae feed on the surface of early-stage developing kernels (Ding and Lamb 1999), resulting in kernel shrivelling and reduction in quality and quantity of yield (Miller and Halton 1961; Olfert *et al.* 1985). Chemical control of wheat midge (Elliott 1988*a*, 1988*b*) is not easy because the recommended application window, the heading growth stage of wheat, is short. Application after this period may kill egg-larval parasitoids of wheat midge and also may adversely affect other natural enemies such as carabid beetles (Elliott 1988*b*; Floate *et al.* 1990; Elliott and Mann 1996; Elliott and Mann 1997).

Deployment of resistant wheat lines to suppress wheat midge populations is a goal of researchers. Cultivars antibiotic to the wheat midge (Barker and McKenzie 1996; Ding *et al.* 2000) containing an antibiosis gene, *Sm1* (McKenzie *et al.* 2002), have been developed. The antibiosis is associated with production of phenolic acids which presumably kill wheat midge larvae when they start feeding on newly developing kernels of antibiotic wheat lines (Ding *et al.* 2000). As antibiosis is conferred by one gene, it is expected that after deployment of cultivars antibiotic to the wheat midge, virulent biotypes of wheat midge might evolve (Smith *et al.* 2007). In order to delay the evolution of wheat midge biotypes virulent against *Sm1*, antibiosis can be deployed together with other resistance mechanisms such as antixenosis. Antixenotic lines were found (Lamb *et al.* 2000*a*, 2002*a*) which decrease wheat midge egg density on resistant wheat lines.

The factors involved in antixenosis of wheat lines to the wheat midge might be visual, chemical and physical cues expressed by resistant plants which individually or together affect the oviposition behavior of wheat midge. These factors are poorly understood for wheat midge. The effects of such factors on the oviposition behavior of other cecidomyiids, for example Hessian fly, *Mayetiola destructor* (Say) (Harris and Rose 1990; Harris *et al.* 1993; Morris *et al.* 2000), and sorghum midge, *Contarinia sorghicola* Coq. (Sharma *at al.* 1990; Naik *et al.* 1996), lead to the hypothesis that wheat midge might be affected similarly by these factors in orienting toward host plants, and use similar cues to detect suitable hosts for oviposition. Exploring the effect of visual, chemical and physical factors on oviposition by wheat midge together with observing oviposition behavior of wheat midge may reveal how antixenosis works, speeding the deployment of effective antixenosis against wheat midge. A more thorough understanding of wheat midge oviposition might also contribute to improved methods for screening for resistance in breeding programs.

With the studies of other agriculturally important midges in mind (Harris and Rose 1990; Sharma *at al.* 1990; Harris *et al.* 1993), and the availability of sources of antixenosis (Lamb *et al.* 2002*a*), the following aspects of wheat midge behavior and interaction with its host plant were investigated. Studying the effect of color on behavior of an insect can be useful in designing management strategies for the pest; for example, attractive colors can be used to enhance the efficacy of traps. Wheat midge is attracted to yellow sticky traps more than blue traps in the field (Oakley and Smart 2002). The effect of background color of wheat spikes and the color contrast between spikes and background is unknown. The results of experiments investigating this may reveal if

wheat midges use visual cues in the pre-landing phase of host selection. After landing on host plants, physical and chemical cues, or their interaction, might affect wheat midge oviposition behavior. The physical or morphological traits of wheat spikes, which might be involved in antixenosis, could be used by breeders as phenotypic markers for selection of lines resistant to wheat midge. Morphological traits of spikes of durum wheat, *Triticum durum* L. are not related to antixenosis (Lamb *et al.* 2001). However, there is no research on the relationship between antixenosis and morphological traits of bread wheat, *Triticum aestivum* L., which has one genome, D, more than durum wheat. In the case of chemical cues, resistant host plants may emit lower levels of stimulants or may release deterrents (Woodhead 1983). A study revealed that wheat midge is attracted to wheat spike volatiles (Birkett *et al.* 2004). It is not known whether low egg density on resistant wheat spikes is due to the effect of wheat spike volatiles. The effect of factors mentioned above will be clarified by observing oviposition behavior of wheat midge on susceptible and antixenotic host plants, to assess what cues a female wheat midge detects during host acceptance and oviposition. There is a recent study on oviposition behavior of wheat midge on pre-anthesis and post-anthesis spikes of susceptible and resistant wheat under natural light conditions (Ganehiarachchi and Harris 2007). This study involved a point observation every 5-min through the oviposition period. It revealed that in spite of the small size and nocturnal activity of a female wheat midge, details of her oviposition behavior are observable. They explained the details of oviposition behavior, found that the presence of male midges increases egg density on wheat spikes, and that female midges oviposit more eggs before midnight than after midnight. However, there are no data from continuous observations of the oviposition behavior of wheat midge.

Continuous observation can lead to quantitative comparisons of details of oviposition behavior on resistant and susceptible wheat spikes.

Phenotyping wheat lines and studying the genetic basis of antixenosis may be helpful in determining the number of genes conferring antixenosis and revealing how it is inherited. Genetic linkage, if any, between antixenosis genes and the antibiosis gene *Sm1* may facilitate transferring resistance genes to other lines. Lamb *et al.* 2002*a* suggested that more than one gene confers antixenosis; however, the number of antixenosis genes is unknown. In order to elucidate the genetic basis of antixenosis and the effect of environment on antixenosis, laboratory and field screening tests of a population of related lines, for example a progeny of a cross between a resistant and a susceptible wheat, are needed. Exploring the cues affecting wheat midge oviposition and the possible genetic basis of antixenosis factors will provide a good base for future steps in development of antixenotic lines against wheat midge.

Research Objectives

The main objectives of the research were to explore the interactions between wheat midge and bread wheat with emphasis on oviposition behavior, and to explore antixenosis from the point of view of crop breeding and genetics. Detailed objectives were as follows:

- To determine whether background color affects oviposition rate of wheat midge.
- To determine the effect of wheat spike volatiles on oviposition rate of wheat midge in the laboratory.
- To screen a set of related wheat lines for antixenosis and antibiosis.
- To determine number of antixenosis-conferring genes.
- To determine interaction between antixenosis-conferring gene(s) and an antibiosis-conferring gene.
- To estimate heritability for antixenosis and assess the environment×genotype interaction.
- To evaluate effects of morphological characteristics of wheat spikes on the oviposition rate of wheat midge.
- To evaluate the effect of host-plant distribution on oviposition rate of wheat midge in the laboratory.
- To describe oviposition behavior of wheat midge in detail in the laboratory.

Literature Review

Introduction

Host-plant resistance to insect pests (hereafter called 'resistance') is an important research field which involves cooperation of entomologists, plant breeders and geneticists. The following review concentrates on entomological aspects of resistance with emphasis on insect oviposition deterrence. The first definitions of resistance were presented by Snelling (1941) and Painter (1951). Resistance can be defined as presence of heritable, measurable and relative trait(s) in a variety of a plant species, enabling that variety to experience less damage and injury from one or more insect pests, compared to damage and injury received by other varieties of the same plant species which lack the trait(s).

Different approaches for classifying types of resistance have been proposed (Painter 1951; Müller 1959; Van der Plank 1968). Painter (1951) proposed the first empirical approach that included three different mechanisms of resistance: antibiosis, non-preference and tolerance. Antibiosis enables a resistant plant to reduce an insect pest's survival, development and reproduction. For example, high constitutive and induced levels of phenolic acids of antibiotic spring wheat lines are associated with high larval mortality of orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) (hereafter called 'wheat midge') (Ding *et al.* 2000). Non-preference, also called 'antixenosis' (Kogan and Ortman 1978), refers to the ability of a plant to reduce oviposition or feeding by an insect pest because it lacks a stimulant or possesses deterrent and/or repellent traits, arising from chemical or morphological characteristics of the plant. For example, leaf pubescence of antixenotic wheat lines greatly reduces

oviposition rate of Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae) (Roberts *et al.* 1979). Tolerance refers to the ability of a resistant plant variety to withstand damage by an insect population which would cause greater injury on susceptible varieties of the same plant species, under similar environmental conditions and similar amounts of feeding activity by an insect. Tolerance has no effect on the behavior or physiology of the insect, at similar insect densities. For example, wheat lines tolerant to Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae), has less dry-plant-weight loss and also less chlorophyll loss compared to non-tolerant lines (Flinn *et al.* 2001).

Examples of variation in responses of crop lines to insect species' attack were reported as early as the 1790s, e.g. the 'Underhill' wheat variety resistant to Hessian fly (Havens 1792). In the early 1800s, 'Winter Majetin' apple cultivar was resistant to wooly apple aphid, *Eriosoma lanigerum* (Hausmann) (Homoptera: Aphididae) (Lindley 1831), and in the early 1900s, American grape varieties were resistant to grape phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Homoptera: Phylloxeridae) (Bioletti *et al.* 1921). Resistance has been an economical, effective, environmentally-friendly and persistent approach for managing insect pest problems (Smith *et al.* 1999; Sharma and Oritz 2002). Deployment of resistant varieties is more effective if the target pest is the major pest of the host plant and the period of the pest's occurrence is short. However, limitations or incompatibilities of resistant plant deployment may include adverse effects on natural enemies, increased plant susceptibility to other insects, toxicity to humans, evolution of virulent insect biotypes and the time needed for developing resistant varieties (Sharma and Oritz 2002).

Currently, deployment of resistant varieties of crops is considered to be an important element in managing human food production systems. Among cereals, wheat, *Triticum* spp. (Poaceae), is an important crop. Wheat is one of the main nourishment sources for the world's population (Johnson *et al.* 1978) because it can be grown across a wide range of environments, offers a good yield, is easily transported and stored, and is used to produce a large variety of products and foods (Briggle and Curtis 1987).

Ancestral species of modern common wheat, *Triticum aestivum* L., were domesticated in the 'Fertile Crescent' of the Middle East 9,000 years ago (Harlan and Zohary 1966; Simmons 1987). Selection of wheat by farmers, in addition to the natural hybridization of species of *Triticum*, with different genomes, gradually resulted in the evolution of modern wheat varieties. According to information collected by FAO in 2005, the major wheat-producing countries are China, India, the United States of America, Russia, France and Canada (FAOSTAT 2005). At present, United States of America is the leading exporter of wheat (USDA 2008).

The genetic resources of wheat, i.e. wheat germplasm, are mostly stored at gene banks such as the International Maize and Wheat Improvement Center (SINGER 2007), International Center for Agricultural Research in the Dry Areas (SINGER 2007) and the U.S. National Plant Germplasm System. The latter has one of the largest collections of crop genetic resources with more than 470,000 accessions (USNPGS 2007). Researchers can acquire and evaluate this germplasm for purposes such as finding resistance sources against pests, creating new hybrids and improving food supply quality and quantity. Once found, desirable traits can be transferred to elite crop genotypes and deployed in agricultural systems.

The use of resistant genotypes to control the major pests of wheat has been reviewed recently (Berzonsky *et* al. 2003). Some of the major insect pests of wheat for which resistant varieties have been deployed or are being developed include Russian wheat aphid, wheat stem sawfly (*Cephus cinctus* Norton, Hymenoptera: Cephidae), Hessian fly and wheat midge. In 1946, deployment of the first wheat stem sawfly-resistant wheat line, which had solid stems filled with pith (Kemp 1934), made a great progress toward controlling *C. cinctus* (Stoa 1947). Since then, many wheat lines resistant to this pest have been deployed and have reduced the severe economic damage of this pest (Cook *et al.* 2004). The most effective method for controlling Hessian fly is deployment of resistant lines. The search for lines resistant to this pest began in 1914 (Dean and McColloch 1915) and was followed by the first deployment of a Hessian fly-resistant line in 1928 (Painter *et al.* 1931). However, virulent biotypes of Hessian fly have evolved (Painter 1930; Harris *et al.* 2003), which has made researchers look for diverse resistance sources and genes to effectively control different biotypes of this pest. Since then, 31 genes, symbolized as *H1* (Nobel and Suneson 1943) to *H32* (Sardesai *et al.* 2005), have been found in different plant species, mainly *Triticum* spp. (Gallun and Reitz 1971; Hatchett *et al.* 1981) and *Secale* spp. (Hatchet *et al.* 1993), and been used in development of Hessian fly-resistant lines. In the case of wheat midge, screening of wheat lines revealed some lines having antibiosis (Ding and Ni 1994; Ding *et al.* 2000; Lamb *et al.* 2000*a*; McKenzie *et al*. 2002) and some having antixenosis (Ding and Lamb 1999; Smith and Lamb 2001; Wise *et al.* 2001; Lamb *et al.* 2002*a*) whose mechanism is unknown. Finding the traits conferring antixenosis would be useful in managing wheat midge.

Orange wheat blossom midge: importance, biology, and control methods

Importance

Gall midges or gall gnats (Diptera: Cecidomyiidae) include many insect species, a few of which are predacious. Most are phytophagous, and their feeding activities sometimes result in an abnormal plant growth called a gall. Examples of economically important crop-pest cecidomyiids are rice gall midge, *Orseolia oryzae* (Wood-Mason); Hessian fly (Harris *et al.* 2003); orange wheat blossom midge (Lamb *et al.* 2000*a*); and yellow wheat blossom midge, *Contarinia tritici* (Kirby) (Borror *et al.* 1989). The latter is a wheat pest found in Europe (Kirby 1798; Barnes 1928) but not in North America. The *C. tritici* adults emerge mostly during June to early July, which usually coincides with the booting stage of winter wheat. Soon after emergence, mating takes place close to the soil surface, as males remain down in the vegetation. Mated females are short lived, usually less than 24 hours, and on sultry evenings lay groups of eggs on the inner surface of glumes or on the anthers of host plants (Barnes 1956). The gregarious larvae are found on the kernel top brush and feed on kernel sap, resulting in whole kernel loss. During humid conditions, full grown larvae wriggle out of spikelets and drop to the soil where they bury themselves and pass their diapause in cocoon (Barnes 1932).

The first report of wheat midge, assuming no misidentification with *C. tritici,* traces back to 1741 in the United Kingdom (Webster 1891). The earliest research on wheat midge, which included some aspects of its biology and host range, was conducted in Europe where wheat midge is considered to have originated (Barnes 1928; Basedow and Schütte 1971). Currently, it is distributed in the northern Great Plains of North

America (Olfert *et al.* 1985; Lamb *et al.* 1999), parts of Europe (Oakley *et al.* 1998), China (Ding and Ni 1994) and Japan (Yuasa 1936).

In Canada, wheat midge was first reported in 1819 near Quebec City (Sanderson 1915). However, according to Barnes (1956), the first report in Canada traces back to 1828 in eastern Canada. Wheat midge was probably introduced from Europe to North America with wheat midge-larvae-infested seeds (Barnes 1956). Since then, wheat midge developed and established in Canada, especially in the Prairies, and was observed in Manitoba for the first time in 1901 (Fletcher 1902) followed by a second report in 1954 (Barker 1984). In 1983, the first severe wheat midge-outbreak occurred in western Manitoba and eastern Saskatchewan, causing $30 million losses in spring wheat (Barker 1984; Olfert *et al.* 1985). This was followed by another outbreak in 1993 in the Prairies, North Dakota and Minnesota. In Canada, it is a key pest of wheat in Manitoba and Saskatchewan (Olfert *et al.* 1985; Lamb *et al.* 1999).

Economic loss due to wheat midge results from the kernel-feeding activity of larvae, causing kernel shrivelling and preventing subsequent normal kernel fill, resulting in yield reduction (Olfert *et al.* 1985), decreased seed germination and seedling vigour (Miller and Halton 1961), deteriorating grain quality (Miller and Halton 1961; Dexter *et al.* 1987; Helenius and Kurppa 1989) and downgrading of harvested wheat. Compensation for wheat midge damage was found in some wheat lines screened by Basedow and Schütte (1973) in Germany. However, compensation was not observed in wheat lines screened by Lamb *et al.* (2000*b*). Depending on wheat prices, insecticide-application expenses and loss of damaged seeds during combine harvesting, the economic

threshold for Canadian spring wheat is 4% to 10% infested seeds before harvest (Lamb *et al.* 2000*b*).

Biology

The principle hosts of wheat midge are common wheat, *T. aestivum* L.*,* and durum wheat*, Triticum durum* L., mostly with spring growth habit in the northern part of agricultural areas of North America, and winter growth habit in Europe and southern North America (Wright and Doane 1987; Wise *et al.* 2001). However, rye, *Secale cereale* L. (Barnes 1956; Basedow 1972); some wild grasses (Barnes 1932) and occasionally barley, *Hordeum vulgare* L. (Reeher 1945; Kurppa 1989; Wise *et al.* 2002) also are infested by wheat midge.

Wheat midge biology can be summarized as follows. Adults emerge from the soil in early summer and initially remain near the soil surface (Reeher 1945; Pivnick 1993). Males are smaller than females, survive for 2-4 days (Pivnick 1993) and mate quickly with females as they emerge. Females attract males by extending the abdomen and ovipositor to release a sex pheromone (Pivnick and Labbé 1992; Gries *et al.* 2000). Pheromone release intensity increases during evening and night compared to the daytime (Pivnick and Labbé 1992). Females and males stay on the lower parts of host plants during the day (Borkent 1989; Pivnick and Labbé 1993). In the evenings, when wind speed is less than 10 km/h and temperature is above 14°C (Pivnick and Labbé 1993), mated females start flying to find hosts at a suitable growth stage for oviposition (Zadoks growth stages 52-60: Zadoks *et al.* 1974); i.e. the inflorescence has started emerging from flag leaf sheath but has not reached anthesis (Basedow and Schütte 1973; Elliott and

Mann 1996; Ding and Lamb 1999). When above crop level, the wheat midge flies downwind, and within the crop it flies both upwind and downwind (Basedow 1977*a*). When the temperature is above 10°C (Pivnick and Labbé 1993), the wheat midge oviposits one to several eggs (Barnes 1956; Mukerji *et al.* 1988; Smith and Lamb 2001) in spikelets of wheat spikes. Females oviposit on different spikelet parts, mainly on the upper, inner surface of glumes but also in florets and on the rachis (Smith and Lamb 2001). A wheat midge female may lay about 60-100 eggs (Reeher 1945; Pivnick and Labbé 1992, 1993; Smith and Lamb 2004) during its five- to seven-day adult stage (Basedow and Schütte 1973; Pivnick and Labbé 1992, 1993). A significant difference in oviposition preference among upper, middle and lower sections of wheat spikes are observed (Smith and Lamb 2001). The middle and upper sections of host spikes are preferred for oviposition (Smith and Lamb 2001). Wheat midge females produce unisexual progenies (Smith and Lamb 2004), probably as a result of a cytogenetic mechanism common in cecidoimyiids (White 1950).

Four to seven days after oviposition, eggs hatch (Mukerji *et al.* 1988). Barnes (1932) observed that solitary larvae crawl toward the kernels' surface, where they establish and start feeding on newly developing kernels. The feeding mechanism is not known, but the feeding does not result in production of galls. At least 11 larvae can mature on a single seed (Lamb *et al.* 2000*b*). Wheat midge has three larval instars for which morphological descriptions are presented in detail (Gagné and Doane 1999). After about 14 days, the second instar larva becomes fully fed, moults into a non-feeding third instar which retains the exuvium of the second instar, probably for decreasing water loss. The third instar stays in the spikelet until wet or humid weather arrives (Hinks and Doane

1988; Gagné and Doane 1999) after which it sheds the exuvium, crawls out of the spikelet and drops to the soil. This happens during August in the Canadian Prairies (Lamb *et al.* 1999). Lack of precipitation at this time may delay larvae departure from the spikelet (Golightly 1952; Basedow and Schütte 1982). On the soil, larvae burrow to a depth of a few centimetres (Golightly 1952), where they diapause, mostly as larvae in overwintering cocoons (Barnes 1956). Unlike *C. tritici*, there is not autumnal emergence (Barnes 1956). Lack of moisture in spring may increase the larval diapause period, so they may wait for the next spring to pupate (Basedow and Gillich 1982). In a laboratory experiment of continuous exposure of larvae to soil temperature of -5 to 2.5ºC, larvae survived at least 500 days (Wise and Lamb 2004). Barnes (1952) reported a rare case of prolonged diapause of 12 years. Increasing temperature and humidity in the following spring breaks diapause of larvae (Basedow 1977*b*). When the soil is moist, the larvae crawl up to the soil surface and make an emergence hole for the adult, and then pupate (Basedow and Gillich 1982; Oakley 1994). Wheat midge adults emerge three to four weeks after pupation, which occurs during mid-June in Europe (Barnes 1956; Basedow and Schütte 1982), and during July in the Canadian Prairies (Lamb *et al.* 1999), coinciding in both places with the booting and heading stages of wheat. In Canada, the length of the emergence period is mostly from the end of June to the end of July (Lamb *et al.* 1999).

Control methods

Cultural, chemical and biological controls are important methods for managing wheat midge populations. In addition, resistant varieties have been developed recently.

Each of these approaches has its own advantages and disadvantages and they can be integrated pattern to bring about effective control of wheat midge.

Cultural control was one of the first approaches used to control wheat midge. Deep ploughing was hypothesized to bury wheat midge larvae deep in the soil, so that larvae could not reach the soil surface to pupate and emerge as adults. However, it was hypothesized that this might result in emergence of adult midges over a wider range of time, due to burying the cocooned larvae to different depths, so whether a late or early maturing line of wheat is grown, it will be infested (Barnes 1956). Crop rotation results in absence of a suitable host at the emergence site of the pest, forcing its dispersal. It can result in pest population suppression by decreasing pest colonization. Rotation was found to be useful for controlling wheat midge (Barnes 1956) and rotating wheat with oilseed crops is recommended in the Prairies (MAFRI 2007). The effectiveness of crop rotation depends on whether all neighbourhood fields are treated the same way. If rotation is applied in some fields and not applied in the others, the wheat midge population will not decrease, as host plants would be available for the pest. Seeding earlier-maturing cultivars and changing seeding date (Kurppa 1989) might be effective in partially controlling wheat midge. Although changing seeding date might be practical and effective in Europe, it is not practical in the Canadian Prairies, due to the short time-window of suitable weather for cereal seeding and growth. Wheat seedlings may encounter cold weather if seeded earlier than usual in the Canadian Prairies. Moreover, coincidence of adult emergence date with heading date of wheat, during mid-June in Europe (Barnes 1956) and during mid-July in North America (Lamb *et al.* 1999),

suggests that this pest has the ability to adapt itself to variation in timing of the host's susceptible stage.

Chemical control can be considered as one of the fundamental elements in managing crop pests. The first studies of chemical control of wheat midge were carried out during the first half of the twentieth century (Mühlow and Sjöberg 1937; Wahlin 1949; Barnes 1952; Mullin *et al.* 1952; Barnes 1955). More detailed experiments done in Finland indicated that application of pyrethroids such as deltamethrin and permethrin, and also organophosphorus compounds such as fenitrothion and parathion at the heading stage of wheat and at emergence time of adult wheat midges had a mean efficacy of 70 to 80% (Kurppa 1988; Kurppa and Husberg 1989). Further research indicated that among several insecticides that were screened for their efficacy to control wheat midge, the one with the active ingredient chlorpyrifos, which has a high vapour pressure, was able to penetrate deep enough into different parts of the spikelet and control wheat midge eggs and larvae effectively (Elliott 1988*a*). Despite these useful results, chemical control of wheat midge is not easy because the recommended application window, which is the heading stage of wheat, is short. Application after this period may kill egg-larval parasitoids of wheat midge and also may adversely affect other natural enemies, such as carabid beetles (Elliott 1988*b*; Floate *et al.* 1990; Elliott and Mann 1996, 1997).

Natural enemies often play an important role in controlling crop pests. Two important hymenopteran parasitoids of wheat midge are *Macroglenes penetrans* (Kirby) (Hymenoptera: Pteromalidae) and *Leptacis tipulae* (Kirby) (Hymenoptera: Platygastidae). The egg parasitoid, *M. penetrans*, is active in Canada. This small wasp, 1-2 mm long, begins emergence from its pupa several days later than its host population. The wasp

searches for wheat midge eggs, followed by the laying of a single egg inside each encountered wheat midge egg. The eggs of the wasp and wheat midge hatch at about the same time. Then the larval wasp develops to the second instar inside the wheat midge larva. The larval wasp remains dormant within the overwintering wheat midge larva until spring when the wheat midge larva is preparing to pupate. At this time, the wasp larva finishes consuming the wheat midge larva, pupates and emerges (Beirne 1971; Doane *et al.* 1989). In other research, parasitism rate ranged from 15% to 48% (Smith *et al.* 2004*a*). The long-term average parasitism rate was 33% in Saskatchewan (Olfert *et al.* 1999). Wise and Lamb (2004) found that wheat midge larvae that do not emerge after the first cold exposure are mostly parasitized.

The egg-larval parasitoid, *L. tipulae,* is active in Europe. Its egg is laid inside the wheat midge egg but remains dormant while the wheat midge larva feeds and overwinters. When the wheat midge larva becomes active in the spring, the wasp egg hatches and the parasitoid larva consumes larval tissue of the wheat midge, pupates within the wheat midge larval skin and later an adult wasp emerges (Elliott and Mann 1996).

Other parasitoids of wheat midge have been reported. *Isostasius punctiger* (Nees) (Hymenoptera: Scelionidae) was reported to be an egg parasitoid in the Netherlands (Doeksen 1938). In the northwest USA, an egg parasitoid, *Inostemma horni* (Ashmead) (Hymenoptera: Platygastidae), was observed by Reeher (1945). *Platygaster tuberosula* (Kieffer) (Hymenoptera: Platygastidae), which was found first by Doeksen (1938) in the Netherlands, as a larval parasitoid of *C. tritici*, shows a high potential for establishing in Canada and affecting the wheat midge population (Olfert *et al.* 2003).

A few wheat midge predators have been reported. For example, the mite, *Anystis vitis* (Schrank), attacks an adult (Klee 1936); the mite, *Trombidium* spp., sucks the abdomen of a female (Barnes 1932); and the thrips, *Haplothrips aculeatus* (Fabricius), eats eggs (Yuasa 1936). In Germany (Basedow 1973), the United Kingdom (Holland and Thomas 2000), and Saskatchewan, Canada (Float *et al.* 1990), carabid predators may attack the wheat midge larva when it returns to the soil surface for diapause. A fungal pathogen, *Entomophthora brevinucleata* Keller and Wilding (Zygomycetes: Entomophthoraceae), was found on cadavers of wheat midge (Keller and Wilding 1985).

The importance of resistant varieties has encouraged researchers, especially in North America, to use this approach as a potentially efficient method in controlling wheat midge. Fletcher (1888) reported that in the late 1800s, a farmer from eastern Canada found one of his winter wheat lines to be less damaged by wheat midge; moreover, another winter wheat line called 'Democrat' was found to be less damaged by wheat midge than other cultivars. It is not clear if these instances were related to real resistance mechanisms or were just host-escape. In early scientific investigation on susceptibility of different varieties of wheat to wheat midge in Europe, the reason for observed differences in infestation levels of studied wheat lines was related to degree of coincidence of midge-emergence date with heading date of wheat lines (Åkerman 1917; Tedin 1917). They reported that awned and awnless varieties are equally susceptible to wheat midge attack. However, in a later study (Lindblom and Mühlow 1932), an awned variety was reported to be less damaged. Later experiments (Rademacher and Klee 1936) on the susceptibility of some German wheat lines, some with winter and some with spring growth habit, did not show any line with a high resistance level. Basedow and Schütte (1974) reported a

few lines, which relative to other cultivars, received less wheat midge damage. Winter wheats with resistance to wheat midge were identified in China (Ding and Ni 1994; Sun *et al.* 1998).

Research done in Canada identified antibiosis in some spring wheat lines derived from winter wheat cultivars obtained from the United States (Barker and McKenzie 1996). More experiments indicated that a single, partially dominant gene, *Sm1*, confers antibiosis in North American wheats (McKenzie *et al.* 2002). Antibiosis in China and North America is associated with increased levels of phenolic compounds. However, the physiological process of antibiosis may not be the same in Chinese and North American wheats (Ding *et al.* 2000). Some wheat lines with *Sm1* have been approved for registration in Canada, but no antibiotic cultivar has been deployed yet. As antibiosis is conferred by one gene, *Sm1*, it will exert high selective pressure on wheat midge after deployment of antibiotic cultivars. This may result in the development of virulent biotypes of wheat midge, eventually making the available antibiosis source useless. Identification of antixenosis in some spring wheat lines, some of which also exhibited antibiosis, increased the possibility of controlling wheat midge more effectively (Lamb *et al.* 2000*a*). In laboratory choice and no-choice tests, the most antixenotic lines received respectively 10% or less and 20% or less oviposition rate compared to susceptible lines. In experimental field plots, these deterrent lines reduce wheat midge infestation at least 50%. It was suggested that more than one gene control antixenosis (Lamb *et al.* 2002*a*). Currently, 10-20% of the harvested seeds are damaged by wheat midge (Lamb *et al.* 1999); a 50%-reduction in oviposition rate in the field can decrease the damage to 5-10% of the harvested seeds (Lamb *et al.* 2000*b*). The durum wheat line, 'Kahla', was

identified to be antixenotic, with oviposition reduced by up to 80% in the laboratory and up to 70% in the field. This antixenosis is not associated with any of the 12 morphological characteristics of durum wheat spikes that were measured (Lamb *et al.* 2001).

To delay evolution of virulent biotypes of wheat midge, two main approaches can be considered. In the first, antibiosis can be applied together with antixenosis. The efficiency of this approach is based on the assumption that pyramiding two types of resistance may delay evolution of virulent biotypes of a pest (Gould 1986). Moreover, its success depends on the two resistance mechanisms being independent genetically and/or physiologically. The second approach is based on maintaining adequate levels of avirulence genes in the wheat midge gene pool by deploying susceptible wheat as a refuge together with resistant wheat. Wheat midges developing on susceptible lines may mate with virulent midges that develop on resistant lines, producing wheat midges heterozygous for virulence. Based on the assumption that virulent alleles are recessive, the heterozygote wheat midges will be susceptible (Smith *et al.* 2004*a*). Wheat midge females distribute their eggs among many plants and in small groups, so we can assume that virulent and avirulent wheat midges will be distributed randomly in fields of mixed susceptible and resistant wheat lines. This makes the refuge approach more feasible. Egg-hatch failure which was observed in some spring wheat lines to be up to 80% (Lamb *et al.* 2000*a*), is another potentially beneficial area to be explored for control of wheat midge. Managing crop pests using resistant varieties of crops requires close cooperation among entomologists, breeders and geneticists. They have to screen more lines and look for new sources of resistance. This keeps them one step ahead of crop

pests. For example, in the case of wheat midge, investigations on some ancestral and modern wheat lines (Wise *et al.* 2001) indicated that there are some promising sources of resistance in some ancestral wheats such as *Triticum spelta* L. and *Triticum dicoccoides* L., which share the A and B genomes with modern hexaploid wheat. Screening more lines representing diverse backgrounds and origins may reveal beneficial sources of resistance to pests, increasing the efficiency of pest management programs.

Factors influencing oviposition behavior of insects

Herbivorous insects, some of which are important crop pests, are attracted to their host plants for feeding, mating and/or oviposition. Generally, the temporal sequence of host utilization by an insect herbivore is categorized into four major steps: finding host-plant habitat by cues such as light, wind, gravity, temperature and humidity; finding a host plant by physical and/or chemical stimuli; accepting the plant as a suitable host by probing; and detecting the host plant's nutrient level, and presence of any toxin (Panda and Khush 1995). These steps are influenced by environmental, physical, visual and chemical factors. In addition, the physiological status of the female insect plays an important role. These factors are reviewed below.

Environmental factors

Insects and their host plants are influenced by environmental factors such as wind, temperature, light, precipitation and soil nutrients, most of which cannot be controlled by humans. However, varying environmental conditions must be considered in studying

insect-plant relations to find appropriate methods for controlling insect pests under different geographic or environmental conditions.

Wind affects insect-flight initiation (Kennedy 1990), flight manoeuvres (VanWoerkom *et al.* 1983), orientation to host-plant odour plumes (Elkington and Cardé 1984), and release rate and concentration of plant odours (Keller 1990). In the laboratory, female Hessian fly stays for a longer period on a wheat spike at high wind speeds, compared to low wind speeds. The on-spike activities of Hessian fly are not affected, so females lay more eggs on wheat spikes at high wind speeds (Withers and Harris 1997). The movement of apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), in the laboratory, decreases as wind speed increases. However, this decline is attenuated when air carries apple volatiles (Aluja *et al.* 1993). Insects may change their flight direction at different wind speeds, which may help them to disperse further. For example, the black bean aphid, *Aphis fabae* Scopoli (Homoptera: Aphididae), takes more vertical flights in windy weather (Haine 1955). Wheat midge starts to fly at wind speeds less than 10 km/h (Pivnick and Labbé 1993). Wheat midge usually flies in or above the crop canopy (Pivnick and Labbé 1993). It was suggested that this behavior may help wheat midge to disperse considerable distances. This may an explanation for the widespread distribution of wheat midge in southern Manitoba, and also for the similar distribution of wheat midge infestations between field edges and interior (Lamb *et al.* 1999). In field studies done in Germany, it was found that *S. mosellana* that flies above the canopy tends to fly downwind; and within the canopy they are found flying upwind as well. They had already laid some eggs whether they were flying upwind or downwind. This behavior

might be more energy-saving than flight against the wind while seeking host plants (Basedow 1977*a*).

Temperature may affect the physiology and behavior of insects and their host plants. For example, it may affect host-plant suitability for herbivore and host-plant response to pest injury (Tingey and Singh 1980). In addition to the effect of temperature on plants, the effects of constant and alternating temperatures on behavior of insects should be considered. Taking temperature effects into account may result in more reliable outcomes of screening of crop germplasm for pest management programs. For example, loss of host plant resistance to Hessian fly with increasing temperature was reported (Cartwright *et al.* 1946). Increases in ambient temperature decrease the post-mating pre-oviposition time of Hessian fly (Harris and Rose 1991). In the case of sorghum midge, *Contarinia sorghicola* Coq. (Diptera: Cecidomyiidae), the susceptibility of some antibiotic resistant lines decrease with increase in minimum and maximum field temperatures, while the susceptible lines show little interaction with this factor (Sharma *et al.* 2003). No work has been done on the effect of temperature on wheat midge-host plant relations. However, studies on its life cycle revealed that the range of temperature for mating is narrow (Pivnick 1993). The optimum temperature for mating was is 19ºC in the laboratory. At 17ºC and 23ºC, no mating activity was observed (Pivnick 1993). Flight activity and oviposition by wheat midge begins when air temperature exceeds 14ºC and 10ºC, respectively (Pivnick and Labbé 1993). These findings indicate that when screening plant germplasm, the appropriate environmental conditions must be provided for an insect to achieve reliable outcomes.

Light also plays an important role in regulating and triggering many physiological processes and behavioral activities. Wheat midge oviposition behavior takes place at the beginning of the scotophase, i.e., the dusk period of the evening (Pivnick 1993). Wheat midge does not lay eggs during the day in the field or when light intensity is high in the laboratory (Pivnick 1993). Providing suitable light conditions in the laboratory is necessary for studying wheat midge behavior and also for screening crop germplasm.

Physical factors

Physical cues or factors which may be detected by insects after landing on plants include the plants' morphological characteristics such as the length and thickness of structures, waxiness and pubescence. In resistant lines, physical factors may have deleterious effects on insect eggs, larvae, or adult insect health and behavior. The adult insect may avoid oviposition on resistant lines and may lay eggs on susceptible lines which lack the deleterious factors. Identifying the resistance-conferring physical factors of crop germplasm can help breeders to transfer these traits into agronomically suitable lines. Sometimes, physical traits do not directly confer resistance but are genetically linked to some other resistance-conferring traits. In this case, these factors can be used as indirect selection criteria by breeders to assist in the selection process for resistant crop lines. For example, selection for sorghum midge-resistant lines was suggested to be successful based on glume, lemma, lodicule and anther length (Sharma *at al.* 1990). Short glume length and short ovary girth of some sorghum lines were linked to resistance in sorghum lines (Naik *et al.* 1996).

The relationships between antixenosis and morphological traits of the host plants of wheat midge have been studied. Wheat lines susceptible to wheat midge are free-threshing and have compact spikes and hulless kernels compared to resistant lines which have tight glumes and less compact spikes (Wise *et al.* 2001). However, there is no difference in wheat midge infestation between hulless and hulled barley lines (Wise *et al.* 2002). One of the reasons for low wheat midge infestation of barley compared to wheat is enclosure of barley spikes by the flag leaf during the growth stage susceptible to wheat midge (Wise *et al.* 2002). Studies on durum wheat identified no relationship between oviposition deterrence and some of the morphological characteristics of spikes of durum wheat lines. In the resistant durum lines, oviposition deterrence might be conferred by fine-scale structural features or surface chemicals (Lamb *et al.* 2000*a*).

Plant-surface pubescence may affect oviposition behavior of insects. For example, female Hessian flies are significantly more restless on pubescent wheat lines compared to glabrous lines (Roberts *et al.* 1979). Pubescent lines of wheat greatly reduces the oviposition rate of cereal leaf beetle, *Oulema melanopus* L. (Coleoptera: Chrysomelidae) (Gallun *et al.* 1973). Legume pod borer, *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae), lays fewer eggs on pubescent wheat lines compared to glabrous lines (Oghiakhe 1995).

Detection of leaf surface structures such as surface pubescence by a female insect may be done by sensilla located around the tip of the ovipositor. For example, the decision to oviposit by limabean pod borer, *Etiella zinckenella* Treitschke (Lepidoptera: Phycitidae), is triggered by mechanical stimulations of receptors at the tip of the ovipositor (Hattori 1988). Ovipositor-dragging on plant surfaces may precede oviposition

which may indicate that the female insect is probing plant traits such as physical, chemical or both traits. Observation of this behavior may help identify physical factors related to resistance.

Visual cues

Most insects can perceive visual cues such as shape, color, distance and movement of objects (Thorsteinson 1960). The visual information obtained can help insects during the pre-oviposition period, when insects search for the host plants' habitat and suitable host plants for feeding and/or oviposition (Thorsteinson 1960).

The shape of objects can be attractive or deterrent to insects (Harris *et al.* 1993). Objects similar to the host plant of an insect are usually attractive. It is inferred that during the evolution of insect-host interaction, vision and orientation behavior has adapted to the shape of the host, increasing the probability of finding an appropriate host plant. An advantage of knowing the effect of the shape of objects on insect behavior is the application of appropriately shaped color-traps in insect pest management programs. For example, cross traps catch more female cabbage root flies, *Delia radicum* L. (Diptera: Anthomyiidae), than vertical or horizontal rectangular traps (Tuttle *et al.* 1988). Vertical objects are more attractive than horizontal ones for apple maggot flies, *R. pomonella* (Moericke *et al.* 1975).

Color is important mostly for diurnal insects and to some extent for crepuscular ones. Insects perceive color through reflectance of light from objects. The color of most plants ranges between pale green and dark green. Objects with reflectance wavelengths close to green, such as yellow, are usually more attractive than objects with other colors.

So, by using visual cues, insects increase their chance of finding a host. Sometimes the color of a plant indicates if the plant is in a suitable condition for the pest's colonization. Different-color races of a host plant species may bring about different responses in an insect pest. For example, red cotton plants are less attractive to the boll weevil, *Anthonomus grandis* (Boheman) (Coleoptera: Curculionidae), than green cotton plants (Maxwell 1977). Red Brussels sprouts are less attractive than green ones for *Pieris rapae* L. (Lepidoptera: Pieridae) (Dunn and Kempton 1976). Studying the effect of color on behavior of an insect can be useful in designing management strategies for the pest; for example, attractive colors can be used to increase the efficacy of traps. Sorghum midge is attracted to yellow, red and white more than blue and black (Wiseman *et al.* 1972; Sharma *et al.* 1990). Wheat midge is attracted to yellow sticky traps more than blue traps in the field (Oakley and Smart 2002). Green leaf models are more attractive than white models for Hessian fly (Harris *et al.* 1993).

Chemical cues

Volatile and non-volatile chemical compounds of plants can affect behavior of insects during searching for suitable host plants and also after landing on host plants. These compounds vary among plant species of the same genus, plant individuals, different growth stages and plant parts (Städler 1986). The volatiles may attract or repel insects during the pre-oviposition period. After landing, the chemical cues mostly act either as a stimulant, i.e. induce the insect to probe, feed and/or oviposit more; or a deterrent, i.e. induce the insect to stop probing, feeding and/or ovipositing. If an insect uses chemical cues of plants to detect a host plant, the lack of host-specific chemical cues

marks the plant as a non-host and the insect leaves the plant before starting to feed or lay eggs. Resistant host plants may emit lower levels of stimulants or may release deterrents (Woodhead 1983). In the case of non-feeding adult insects such as wheat midge, detection of oviposition stimulants or deterrents in susceptible and resistant host plants can be useful for breeders in production of antixenotic lines.

An insect either can detect the specific volatiles of the host plant among other volatiles of surrounding plants, or can recognize the appropriate ratio of volatiles of the host plant. The latter was suggested to occur in the case of wheat midge, and also some of the attractive compounds for wheat midge were identified (Birkett *et al.* 2004). Stimulatory and attractive compounds for Hessian fly were found in the host plants (Foster and Harris 1992; Morris *et al.* 2000; Cervantes *et al.* 2002).

Insects perceive chemical cues by chemoreceptors located on the tarsae, antennae, mouthparts and/or ovipositor. For example, *Delia* spp. flies and some butterflies detect chemical cues by foreleg tapping and using tarsal chemoreceptors (Beck and Schoonhoven 1980; Schoonhoven *et al.* 1998). It was suggested that flying Hessian fly female perceives stimulants and recognizes the suitable plant for oviposition by touching the plant surface with antennae, legs and the tip of abdomen (Harris *et al.* 1993).

The physiological age of plant tissues may affect resistance (Tingey and Singh 1980), possibly as a result of chemical differences among plant growth stages. For example, Hessian fly oviposits more on young wheat leaves which contain higher amounts of aldehydes (Morris *et al.* 2000). The oviposition rate of a wheat midge decreases after anthesis of host plants (Elliott and Mann 1996), but the possible chemical basis for the change has not been studied. The presence of anthers and pollen of wheat

has no effect on the oviposition rate of wheat midge (Ding and Lamb 1999). However, the physical removal of anthers and stigmata significantly reduces sorghum midge oviposition rate, which was also observed in male sterile sorghum lines (Sharma *et al.* 1990).

Environmental, physical, visual and chemical factors affecting oviposition behavior of an insect were discussed separately here, but mostly, it is the combinations of these factors that lead to successful host finding and oviposition. Considering all of these factors to explain antixenosis-conferring mechanisms in a plant is required. For example, yellow color, tubular structure and onion volatiles, together, have more effect on oviposition rate of *Delia antiqua* (Meigen) (Diptera: Anthomyiidae) than each factor alone (Harris and Miller 1982). Additive effects of visual and chemical cues also were found in the case of Hessian fly (Harris *et al.* 1993).

Genetic aspects of resistance in plants

Resistance-conferring traits must be heritable to be useful in breeding and insect pest management programs. Studies on inheritance of resistance to pests traces back to the early twentieth century, when the resistance of cotton to leaf blister mite*, Eriophyes gossypii* Banks (Acari: Eriophyidae), was found to be a heritable characteristic (Harlan 1916). Since then, development of quantitative and molecular genetics has revealed the genetic basis of resistance, helping to define the number of genes conferring resistance, revealing the dominance or recessive nature of resistance genes, linkage among genes, and interaction between genes and environments. The importance of these aspects is briefly reviewed below.

Obtaining adequate information on the genetic nature of traits helps breeders to successfully transfer genetic resistance into commercial cultivars (Agrawal *et al.* 1988). Resistance-conferring trait(s) of a plant is controlled by a gene(s) that occupies a specific location, i.e. locus, on a chromosome of the plant genome. The traits are the final expression of the genes.

The genome of a plant consists of several chromosomes and many genes. The wheat genome is one of the largest and most complex of all crops. It has been studied in detail and a nearly-complete genomic map is now available (Somers *et al.* 2004; IWGSC 2007). Bread wheat, *T. aestivum*, is a hexaploid plant and its genome consists of 42 chromosomes and three different ancestral genomes, A, B and D, each containing a set of 7 chromosome pairs. Durum wheat, *T. durum,* is a tetraploid plant, and its genome consists of A and B genomes with 28 chromosomes (Sakamura 1918; Kihara 1924). Many insect-resistance genes have been found in wheat, some of which have been deployed in insect pest management programs. For example, Hessian fly resistance genes have been found mostly in the A genomes of wheat (Gallun and Patterson 1977; Stebbins *et al.* 1982) and D genomes of wheat (Gill *et al.* 1987).

Information on the number of resistance genes in germplasm is useful for entomologists and breeders. Monogenic traits usually confer higher levels of resistance than polygenic ones. Managing monogenic traits is easier than managing polygenic traits in hybrid production by breeders. However, it is predicted that an insect will develop a virulent biotype against a monogenic trait faster than to a polygenic trait (Gallun and Khush 1980). Antibiosis of some soybean lines to the agromyzid stem fly, *Melanagromyza sojae* (Zehntner) (Diptera: Agromyzidae) was found to be controlled by

one dominant gene (Gai and Wang 1998). Antibiosis to corn borer, *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae) is conferred by dominant genes, so improved resistance levels would be easily obtained with selection of studied lines (Cartea *et al.* 2001). Antibiosis to sorghum midge, *C. sorghicola,* was conferred by more than two recessive genes, making gene transfer challenging (Boozaya-Angoon *et al.* 1984). In the case of wheat midge, Lamb *et al.* (2002*a*) suggested that more than one gene may control deterrence in the studied wheat lines.

Sometimes genes are linked to each other, so that they are inherited together and recombination rarely occurs among them. Sometimes a resistance gene is linked with an agronomically unsuitable gene, complicating the breeding processes. However, the resistance gene might be linked to a marker which might be identified easily by molecular methods or which is expressed in obvious and easily identifiable morphological or physiological characteristics. Breeders can use the markers in selection processes for development of resistant lines (Thomas *et al.* 2005).

Breeders search for cultivars which have stable expression of agronomic traits under different environmental conditions. However, it is usually observed that expression of traits in genotypes change across test environments (Allard and Bradshaw 1964). Variability might be related to interactions among genes and environments (Graybill *et al.* 1956). Information about genotype×environment interactions of a group of genetically related lines is important in the determination of the 'heritability' parameter (Nyquist 1991).

In quantitative genetics, 'heritability' is defined as the ratio of total genetic variance to phenotypic variance. The variation among lines of a germplasm regarding a

trait, e.g. resistance to a pest, is called phenotypic variation, which is the sum of environmental variation, genotypic variation and genotype×environment variation. The values for genotypic and genotype×environment variation can be estimated through analysis of variance of the observed results regarding the tested trait. Genotypic variation is the result of the effect of genes. So, high values for genotype effect and low values of environmental effect indicate that the tested trait of the lines is less affected by different environmental conditions, and it is mostly affected by the genes, i.e the trait has high 'heritability'. In plant breeding, traits with high heritability can be improved more rapidly with less intensive evaluation than those with low heritability (Dudley and Moll 1969). Moreover, estimating gene number conferring a trait is more reliable when heritability is high (Park 1977).

Genes conferring resistance to Hessian fly

Deployment of resistance genes has been found to be effective in controlling Hessian fly (Berzonsky *et al.* 2003). Hessian-fly resistance genes confer antibiosis that kills first instars soon after they start feeding. Inheritance of resistance to Hessian fly in wheat was first reported by Cartwright and Wiebe (1936). Since then, 31 resistance genes have been found in *Triticum* spp., *Secale* spp. and *Aegilops* spp. plants (Caldwell *et al.* 1946; Shands and Cartwright 1953; Hatchet *et al.* 1981; Patterson *et al.* 1988; Cambron *et al.* 1995; Delibes *et al.* 1997; Williams *et al.* 2003). The first deployed resistance genes were *H3* in the cultivar 'Dual' in 1955, and *H6* in the cultivar 'Knox 62' in 1962 (Foster *et al.* 1991). The genetic analysis of wheat germplasm, obtained from different geographic regions of the world, indicated that inheritance of antibiosis varies from

recessive to dominant and is controlled with one, two or multiple genes. For example, the antibiosis to Hessian fly biotypes B and D is conferred by one gene in the wheat cultivar 'Luso' (Oellermann *et al.* 1983). Antibiosis against biotype L is monogenicly controlled and partially dominant in 'Marquillo' (Maas *et al.* 1987). Antibiosis to biotype D of Hessian fly is conferred by two genes, *H9* and *H10* in a durum wheat cultivar 'Elva' (Stebbins *et al.* 1982). Antibiosis in the cultivar 'Seneca' is conferred by two independent genes (Amri *et al.* 1990). The effectiveness of deployment of resistant lines for controlling Hessian fly leads us to infer that this control strategy can be applied to suppress wheat midge damage.

Doubled-haploid plants and antibiosis gene against wheat midge

Production of completely homozygous plants is important for crop breeders, because homozygous lines are genetically stable. Moreover, genetic analysis and screening processes for homozygous lines are easier (Schaeffer *et al.* 1979; Choo and Reinbergs 1982*b*). Homozygous plants can be produced by self-pollination over six or more generations. However, this method is time consuming, so that breeders use a faster method, called the 'doubled-haploid' method.

The doubled-haploid method involves production of homozygous lines through doubling the chromosomes of a haploid plant. In this method, the produced homozygous lines are called doubled-haploid lines. Development of 'anther culture' methods (Guha and Maheshwari 1964; Nitsch and Nitsch 1969; Kasha and Kao 1970) and 'chromosome elimination' methods (Barclay 1975; Laurie and Bennett 1988; Inagaki and Tahir 1990; Matzk and Mahn 1994) increased the interest of breeders in developing faster methods

for production of pure lines and haploid plants. 'Chromosome elimination' methods were found to be more reliable than 'anther culture' methods (Kisana *et al.* 1993; Mujeeb-Kazi *et al.* 1995). One of the methods in chromosome elimination is pollination of a plant such as wheat, with a haploid inducer, e.g. pollen of maize, *Zea mays* L. (Poaceae). Maize chromosomes are eliminated after the cross. Haploid embryos are excised and moved to a tissue culture medium treated with chemicals such as 2,4-D and later haploid plants are transferred to soil (Laurie *et al.* 1990). The haploid plants are subjected to colchicine, which causes each single chromosome to double. The plant that is grown from this seed is referred to as a doubled-haploid. The new plant is a genetically-stable homozygous line, and will produce genetically identical progeny (Pienaar *et al.* 1997). Production of doubled-haploid wheat lines helped to identify *Sm1*, the gene conferring antibiosis to wheat midge (McKenzie *et al.* 2002).

Antibiosis to wheat midge was found initially in winter wheat lines from the United States (Barker and McKenzie 1996). The antibiotic lines kill wheat midge larvae through elevated levels of phenolic acids produced after feeding by wheat midge larvae (Ding *et al.* 2000). Research using doubled-haploid populations revealed that antibiosis was controlled by a single, partially-dominant major gene, designated as *Sm1* (McKenzie *et al.* 2002) which is on chromosome 2B (Thomas *et al.* 2001). The antibiotic lines decrease larval density 59-100%, and reduce seed damage by 70-100% compared to a susceptible line (Lamb *et al.* 2000*a*). Antibiosis of this type has not been found in durum wheat (Lamb *et al.* 2001). As the antibiosis is conferred by one partially dominant gene, its detection in homozygous and heterozygous antibiotic lines is reliable. Moreover, *Sm1* has a high heritability, so its incorporation into breeding programs will be efficient and

easy for breeders. Association or linkage of *Sm1* with deterrence-conferring gene(s) has not been found. However, linkage between *Sm1* and leaf rust resistance gene *Lr16* have been found and is used in indirect selection breeding programs (Thomas *et al.* 2005). Genetic analysis of lines that confer antibiosis and also antixenosis, can clarify the linkage, if any.

Discussion

Deployment of insect-resistant crops has considerably decreased yield loss due to pest damage. Deployment of resistant lines is an environmentally-friendly control method, which in combination with other pest-control methods may result in an effective suppression of pest populations. Resistant cultivars are technically easier to manage than using insecticides on susceptible cultivars. Successful results in production of pest-resistant lines need close collaboration among breeders, entomologists and geneticists. For example, entomologists have contributed to the knowledge about two key pests of wheat in most wheat growing regions, Hessian fly and wheat midge through exploring the pests' life cycles, screening lines for resistance to the pests, and elucidating the resistance mechanisms. In collaboration with breeders, Hessian-fly antibiotic lines have been deployed for decades with good results. However, in the case of wheat midge, sources of antixenosis and antibiosis have just been identified. Wheat lines with monogenic antibiosis to wheat midge have just been registered for deployment into commercial production. There is hope that antibiosis will depress the population of wheat midge effectively. However, the pressure on wheat midge population will probably result in evolution of virulent biotypes (Smith *et al.* 2007).

New sources of resistance, which confer antixenosis to wheat midge, have been identified. Antixenosis can reduce initial colonization of wheat midge on wheat and can be considered as the first barrier for wheat protection. Combination of antixenosis and antibiosis together can decrease the opportunity for the evolution of virulent biotypes of wheat midge, and conserve valuable resistance genes. Deployment of wheat midge resistant lines in the Prairies, where most of the wheat in Canada is produced, can substantially decrease farming expenses through reduction of insecticide application. Close collaboration with geneticists is needed to transfer genes into other cultivars, and do related molecular research.

In this research, doubled-haploid wheat lines produced at the Cereal Research Centre, Agriculture and Agri-food Canada, Winnipeg, Manitoba, will be tested in the laboratory and in the field, to identify resistant lines, determine the number of antixenosis-conferring genes, and examine relationships between morphological traits of wheat spikes and antixenosis. Moreover, wheat midge oviposition behavior, the process affected by antixenosis, will be studied in detail. As antixenosis-conferring genes might be affected by environmental conditions, so screening of lines will be conducted both in the laboratory and in the field. The results will clarify the mechanism of antixenosis and help breeders to select antixenotic lines more rapidly. The resistant lines can be used in future hybridizations.

In general, genetic information such as heritability and number of antixenosis genes can help breeders to choose the appropriate breeding method for production of resistant lines.

Laboratory and field tests for discrimination of resistant lines is time consuming, so determining the relationship between a morphological trait of wheat and antixenosis would save time for breeders to detect resistant lines faster by using these traits as 'morphological markers'. The genes conferring morphological traits may also be associated with antixenosis. In the alternate case, the genes for morphological traits might be linked to the other genes that confer antixenosis. If positive results are obtained, molecular analysis must be done to identify the location of genes on chromosomes.

In addition to the effect of morphological traits, the effect of volatiles released by wheat spikes will be determined. It is known that most insects use chemical cues to find and detect suitable host(s). If the volatiles of the available germplasm affect the oviposition rate of wheat midge, chemical analysis may reveal the compounds inducing antixenosis. Later, the genes responsible for these compounds can be identified and transferred to other cultivars.

Behavioral observations will be made to understand the effect of physical and chemical cues on oviposition rate. Analyzing wheat midge oviposition behavior on resistant and susceptible lines can clarify how an insect allocates eggs during host probing and oviposition period. The observations can reveal unknown aspects of wheat midge host finding, host acceptance and oviposition. The results can help in designing appropriate tests for detection of antixenosis mechanisms.

Visual cues, such as color, can affect wheat midge oviposition rate. Different colors might be attractive or repellent to wheat midge. For most insects, yellow is the attractive color. It is our assumption that yellow can be one of the attractive colors to wheat midge. However, there is no information about the deterrent color(s). It is hard to

change the color of the cultivars without affecting their physiology, so information on deterrent colors cannot be applied directly in plant breeding programs; however, this information can be useful in designing appropriate laboratory experiments for line selection tests. Use of inappropriate colors in an experiment may affect wheat midge behavior, which can bring about biased experimental results. Moreover, attractive colors can be used in production of color traps. Application of deterrent colors in color traps, which are used to determine the appropriate time for insecticide application, can bias the result of experiments related to wheat midge population dynamics.

Chapter 1

Plant material and culture of wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae)

Plant material

Plant materials used in this study were 92 doubled-haploid spring wheat lines (Appendix 1) developed from the cross 'Roblin' × 'Key 10' which is designated as 'BD140'. 'Roblin' is a wheat midge-susceptible cultivar (Lamb *et al.* 2000*a*) with high yield and good agronomic traits. 'Key 10' has antibiosis and antixenosis to wheat midge (Lamb *et al.* 2000*a*).

The line 'Key 10' was created by crossing the American winter wheat cultivar 'Augusta', which contains the antibiosis gene *Sm1*, with 'HW Alpha'. Doubled-haploid lines were obtained from F_1 generation. The lines with winter growth habit were discarded and those with spring growth habit were kept for propagation. 'Key 10' was one of the doubled-haploid progeny with spring growth habit.

The doubled-haploid lines described above were produced by researchers at the Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba, using the 'wheat × maize cross' method (Laurie and Bennett 1988). In addition to the 92 doubled-haploid lines, the parents, 'Key 10' and 'Roblin', were included as controls in all experiments. The seeds were kept in labelled envelopes in a refrigerator (4-5°C). In order to have enough seeds for future experiments, seed of each line was increased in a growth cabinet and a greenhouse during winter 2004 and spring 2005.

Wheat plant seeding and culture

In the laboratory, plants were grown hydroponically in tubular plastic pots (Stuewe & Sons, Inc., Corvallis, Oregon, USA), which were 4 cm in diameter at the top and 20 cm high, with five 1-cm holes at the bottom. In a rack (60 × 30 × 17.5 cm, length:width:height) which had a seven-by-fourteen array of 4-cm holes, the clean pots were arranged and filled to within 1-cm from the top with artificial soil, hereafter called soil (Metro-Mix®, Terra-Lite® 2000, Grace Horticultural Products, W. R. Grace & Co. Ltd., Ajax, Ontario, Canada). The soil was compacted with a rod to remove large air pockets.

The code numbers of lines to be seeded were written on the outside of the plastic pots, with a permanent marker. Seeds were put on the soil of the corresponding pots and then the pots were filled to the top with soil. One plant per pot was grown. The pots were arranged in every other hole of a rack to ensure that plants would get enough light at the later growing stages in the growth cabinets. The line series, time of seeding and fertilization dates were written on a plastic label, which was inserted in one of the pots in the rack. The racks were put in clean tubs, transferred into a growth cabinet (Conviron®, Model: E15, Controlled Environment Ltd., Winnipeg, Manitoba, Canada) and the tubs were filled with tap water. The conditions in the growth cabinet were 18 H:6 H L:D, 20ºC during light-on, and 10ºC during the dark period. Light sources in the growth cabinet were fluorescent lamps and incandescent bulbs located on a height-adjustable rack above the plants. One week after seeding, and every two weeks thereafter, fertilizer was added to each tub (15 g of Plant-Prod® 20-20-20 [N-P-K] in 10 litres of water) (Plant Products Company, Ltd., Brampton, Ontario, Canada). Before adding the fertilizer to the tubs, the

racks containing the plants were moved from the tubs, the water in the tubs was renewed, the fertilizer was added, the roots of the plants were trimmed, and the racks containing the plants were moved back into the tubs. When plants needed more space for growth, racks were transferred into bigger growth cabinets (Conviron®, Model: PGW36, Controlled Environment Ltd., Winnipeg, Manitoba, Canada) which had the same environmental conditions as the small cabinets. The senescent leaves were also removed regularly to produce strong spikes and to discourage the growth of powdery mildew. If mildew incidence was high, the cabinets were sprayed with the fungicide propiconazole (Tilt®).

Wheat midge culture

Wheat midges for the experiments were reared in laboratory cultures, providing a continuous supply of adult wheat midges. To prevent selection of a laboratory strain of wheat midge and to keep the genetic structure of the wheat midge population similar to that in the field, the adults reared from field-collected larvae were mixed with the laboratory-cultured adults during laboratory propagation of the wheat midge. To obtain field midge, spikes of 'Roblin' or 'AC Barrie' spring wheat were collected during early August from infested fields. The spikes were transferred to the laboratory, and either dissected under a stereomicroscope (Wild-Heerbrugg®, Wild Leitz Canada, Ltd., Willowdale, Ontario, Canada) or hand threshed to collect the third instars. For hand threshing, the spikes were dried for a week or two at room temperature, then hand threshed and the chaff sieved to screen out the larvae. Collected larvae were divided into lots of 200, and placed on sandy loam in clear plastic 250 ml containers. The soil was

moistened, the containers were closed with snap-on lids, and left at room temperature to let larvae come out of the second instar exuvium and burrow into the soil. After 3 to 4 weeks, the containers were transferred to a 2.5ºC storage room and kept for at least four months, so that larvae could complete their obligatory diapause. Four to 5 weeks before adult wheat midges were required, larvae which had completed diapause were transferred to the laboratory in their containers, the soil was moistened, and the containers were placed in a growth cabinet at 20ºC, 70% R.H. and a photoperiod of 16 H light and 8 H dark (Conviron®, Model: E8L, Controlled Environment Ltd., Winnipeg, Manitoba, Canada). After about 4 weeks, when the first adults started emerging, the lids were replaced with 2-litre clear plastic containers (11 × 34 cm, diameter:height) made from soft-drink bottles with the label and base removed and the top hole blocked with cotton batting. Wheat midge parasitoids usually occurred in the cultures originating from field samples. However, the parasitoids usually emerged after emergence of most adult wheat midges, so parasitoids were easily excluded.

To provide laboratory cultures of wheat midge, 'Roblin' plants with spikes in the susceptible stage for oviposition (emerged from the flag leaf but pre-anthesis) (Zadoks growth stage 57-59: Zadoks *et al.* 1974), were transferred into a controlled-environment room, hereafter called the test room. The test room conditions were 20 ± 3ºC, 45% to 70% R.H. and a 20 h light period including dawn and dusk intervals of 2 h each, during which light intensity changed gradually. The light source of the test room was incandescent bulbs and fluorescent lamps located on the ceiling. The intensity and dimming activity of the ceiling lights was controlled by a programmable electronic device (GRAFIK Eye®, Lutron Electronics Co., Coopersburg, Pennsylvania, USA). In

order to provide more light to the wheat spikes, six 120-cm fluorescent lamps (Sylvania®, Supersaver®, F34CW/SS/ECO, 34 Watt, Canada; Philips®, F34T12/CW/RS/EW, 34 Watt, USA) were positioned on a 135-cm-high iron frame above each cage in which the experiments were done. The above-cage lamps were on for 16 h per day which coincided with the period when the ceiling lamps were at full intensity.

The cage was a 60-cm wooden-frame cube, covered with transparent polyethylene plastic on the outside, and plastic mesh on the inside separated by 2 cm from the plastic. The mesh provided a resting surface for wheat midge, as static electricity and moisture on the plastic could cause the wheat midge to stick to it. The cage had a circular gate (22 cm in diameter) on one side which was used to release the adult wheat midges into the cage. The cage was placed on top of a wooden base frame, 60 × 60 cm square and 65 cm tall, with 4 small wheels. The bottom of the base frame had a basin for holding water. The plants in pots were inserted in the cage base, a 5-mm-thick wooden sheet which had a five-by-five array of 4-cm holes and fixed at the height of 15 cm above the bottom of the basin.

The plants, the inside of the cage and base frame were misted with water because oviposition of wheat midge occurs at high relative humidity. Female and male wheat midges, which were in the emergence bottles in the growth cabinet, were brought to the test room and released into each cage, at a rate of one to two females per spike and one to two males per two females. The dates of cage setup and dismantling and the name of the test were written on tape attached to the cage wall to distinguish it from other cages in the test room. After 2 or 3 nights, the plants were removed from the cage, and the spikes were covered with glycine pollination bags to maintain humidity and prevent eggs from

desiccating. The plants were placed in a rack and transferred to the greenhouse where the rack was placed in a clean tub of water. After 3 weeks the spikes were excised and transferred to the laboratory. The spikes were hand-threshed, as described above, to collect third instar larvae, which were reared to the adult stage as was done for the field-collected larvae.

Chapter 2

The effect of visual and chemical cues on oviposition rate of wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae)

Introduction

Herbivorous insects are attracted to their host plants for feeding, mating and/or oviposition, during which they are influenced by their environment. Exploring the effect of physical, visual and chemical factors and their interaction with insect behavior can be useful for managing crop pests and breeding resistant crops.

Most insects can perceive visual cues such as color. The visual information obtained can help herbivorous insects during the pre-oviposition period, during which insects search for the host plant habitat and suitable host plants for feeding and/or oviposition (Thorsteinson 1960). Color is important mostly for diurnal insects and to some extent for crepuscular insects that are active at dawn and dusk. By using visual cues, insects increase their chance of finding a suitable host. Sometimes the color of a plant indicates if the plant is in a suitable condition for the herbivore's colonization. In some cases, differently-colored races of a host plant species elicit different responses in an insect pest (Dunn and Kempton 1976: Maxwell 1977). Studying the effect of color on behavior of an insect can be useful in designing management strategies for a pest; for example, attractive colors can be used in traps. The effect of color on behavior of cecidomyiids when approaching different objects and host plants has been studied (Wiseman *et al.* 1972; Sharma *et al.* 1990; Harris *et al.* 1993; Oakley and Smart 2002).

Volatile and non-volatile chemical compounds of plants can affect behavior of insects when searching for a suitable host plant and also after landing on host plants. These compounds vary among plant species of the same genus, plant individuals, different growth stages and plant parts (Städler 1986). Volatiles may attract or repel insects during the pre-oviposition period. After landing, chemical cues can act either as a stimulant, i.e. make the insect probe, feed and/or oviposit more (Thorsteinson 1960), or a deterrent, i.e. make the insect stop probing, feeding and/or ovipositing (Dethier *et al.* 1960). If detection of a plant as host is done by perceiving chemical cues by the insect, the lack of host-specific chemical cues marks the plant as a non-host and the insect leaves the plant before starting to feed or lay eggs. Resistant host plants may emit lower levels of stimulants or may release deterrents (Woodhead 1983). In the case of non-feeding adult insects such as wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), quantification of oviposition stimulants or deterrents in susceptible and resistant host plants can be useful for plant breeders to produce antixenotic lines.

The physiological age of plant tissues may affect resistance (Tingey and Singh 1980), possibly as a result of chemical differences among growth stages (Morris *et al.* 2000). The oviposition rate of wheat midge decreases after anthesis of host plants (Elliott and Mann 1996), but the possible chemical basis for the change has not been studied.

The wheat midge is a key pest of wheat, *Triticum aestivum* L. (Poaceae), in the northern Great Plains (Wright and Doane 1987; Lamb *et al.* 2000*b*). Females start ovipositing at dusk, a short time before sunset until dark (Reeher 1945; Pivnick and Labbé 1993; Ganehiarachchi and Harris 2007) on wheat spikes (Mukerji *et al.* 1988). Larvae feed on the surface of early-stage developing kernels (Ding and Lamb 1999),

resulting in kernel shrivelling and reduction in quality and quantity of yield (Miller and Halton 1961; Olfert *et al.* 1985). In addition to the widespread application of insecticide to control this pest (Elliott 1988*a*, 1988*b*), cultivars resistant to the wheat midge (Barker and McKenzie 1996; Ding *et al.* 2000) containing the antibiosis gene *Sm1* (McKenzie *et al.* 2002) have been developed. Antixenotic lines were found (Lamb *et al.* 2000*a*) and research is continuing to explore the genetic basis of antixenosis. The effect of visual and chemical cues on the behavior of the wheat midge can be helpful in scrutinizing antixenosis in wheat lines.

Wheat midge oviposition on wheat spikes increases before anthesis and decreases after anthesis (Elliott and Mann 1996; Lamb *et al.* 2003). It is not known if oviposition suppression at post-anthesis is due to chemical or tactile cues. Studies on sex pheromone of wheat midge (Pivnick 1993; Gries *et al.* 2000), special structures on the antenna of cecidomyiids (Slifer and Sekhon 1971), and research on host selection by insects (Kennedy 1965; Dethier 1982; Nojima *et al.* 2003) such as Hessian fly (Harris and Rose 1990; Foster and Harris 1992) lead to the hypothesis that wheat volatiles (Buttery *et al*. 1985; Birkett *et al.* 2004) affect host finding and oviposition of wheat midge. The only research on the effect of wheat spike volatiles on wheat midge (Birkett *et al.* 2004) showed that wheat midge responds behaviourally to volatiles of host plants. Understanding the interactions among the wheat plant cues and wheat midge can be helpful in managing and controlling this pest in the field.

The objectives of this research were to explore the effect of background color as a visual cue on wheat midge egg density on spikes of spring wheat in the laboratory, and

assess the effect of pre-anthesis and post-anthesis volatiles of spring wheat spikes on oviposition rate of wheat midge in the laboratory.

Materials and Methods

The effect of background color on oviposition rate of wheat midge

Combinations of colors were used in four separate tests: 1) red, yellow, green and blue (5 replications); 2) yellow, green, blue and control (5 replications); 3) black vs. control (7 replications), and 4) red vs. control (5 replications). In the test room, a 60 × 60 × 5-cm block of Styrofoam was placed on the base frame described in Chapter 1. The Styrofoam was covered with black plastic film, and covered with a 5-mm layer of soil, to simulate the presence of soil in the field. Forty spikes of the wheat cultivar 'Roblin' which were at the pre-anthesis stage but were out of the flag leaf were chosen (Zadoks growth stage 57-59: Zadoks *et al.* 1974), and the culms were cut off at 50 cm from the top of the spikes, and all leaves except the flag leaves were excised. Hereafter the excised culm carrying the flag leaf and the spike will be called a spike. The spikes were transferred to the test room, where they were placed in 12-cm-long plastic floral tubes (Floral Piks®, Dakota Plastics Co., Watertown, South Dakota, USA) which had thin extensions for insertion into the Styrofoam. The piks were placed in a bucket of water, filled with water and lidded with snap-on caps, each with a 3 mm aperture at the centre for inserting plant stems. Each spike was cut at a height of 40 cm from the terminal spikelet, while under water, then immediately inserted into the pik under water, to prevent air entrance into the culms, which could have adverse effects on the physiological status of the spikes during the test. When all 40 spikes were ready, 10 spikes were

positioned vertically, touching each other, at each corner and at a distance of 4 cm from the edge of the arena. The space inside a cage is referred to as an arena.

Color folders supplied by Grand & Toy® (Don Mills, Ontario, Canada), with the following factory codes, were used in the experiment: red #99331, yellow #99334, green #99326 and blue #99330. Each side of a color folder was 37 × 31 cm (length:width). For black color, two black 21.5 × 27.9 cm sheets of paper were taped together on their long edges to resemble the shape of the folders. In Test 1, four different colors were used in one cage: red, yellow, green and blue. In order to control for position of the folders, the colors were rotated among different corners of the cage for each replication. The folders were attached to the cage walls so that the middle of the folder was parallel to one corner and each side of the folder faced the adjacent walls with the upper edge 3 cm from the top of the cage. The color folders were fastened by small pins to the corners of the cage. After fastening all folders, the walls of the cage and spikes were misted with water to maintain high relative humidity for wheat midge during the test. The cage was placed on the Styrofoam block and the edges between the cage and the block were sealed with tape to maintain the cage position. In each cage, 40 female and 40 male wheat midges were released (one female and one male midge per spike). The cages were set-up in the afternoons, and each replication was run for three nights. Temperature and humidity level of the test room were checked daily to ensure they remained around 20°C and 70%, respectively. In the morning of the fourth day, the cage was dismantled. The spikes in front of each color folder were collected, labelled, and then dissected under a stereomicroscope at magnifications of 6x to 50x.

The cage set-up date, dismantling date, colors used in the test, number of plant samples for each color, plant variety used, height of plant samples, the treatment color, and number of spikes for each treatment were recorded. Data collected for each spike were spike length (in centimetres), number of spikelets, and number of eggs laid on each spikelet. The spikelets were numbered sequentially from the base of the spike to the terminal spikelet. Eggs were counted on all parts of each spikelet and its corresponding rachis inter-node.

In Test 2, three colors were used: yellow, green and blue. This test was set up the same as Test 1, except that one corner of the cage did not contain any color folder, and the plant samples located in this corner were considered as 'control'. The mesh and plastic cover of the cage behind the control corner provided a pale beige background.

In Test 3, the effect of a black background was explored. The general procedure was the same as for Test 1, except that just 20 spikes were used in each replication: 10 spikes in front of the 'black corner', and 10 spikes in front of the 'control corner' which was diagonal from the black corner.

In Test 4, the effect of a red background was studied. The general procedure was the same as Test 3, except that a red folder was used instead of black sheets.

The spectral reflectance of the color folders were measured by C. Nansen, Texas Tech University, using a hyperspectral spectral camera (PIKA II, Resonon Inc., Bozeman, MT, USA).

Chemical cues and antixenosis

The effects of volatiles released by pre-anthesis or post-anthesis spikes of either the resistant spring wheat line, 'Key 10', or the susceptible spring wheat cultivar, 'Roblin', on the oviposition rate of wheat midge on 'Roblin' or 'Key 10' were explored in four different tests.

Figure 2.1 shows how the apparatus for this experiment was set up. The parts of the system were rinsed with 75% ethanol followed by rinsing with distilled water before setting up the device and also between tests. The base frame was positioned under fluorescent lamps of the test room. A 60 × 60 × 5-cm block of Styrofoam was placed on the base frame, next to an air valve. The plastic tubes used in this experiment were 1 cm in diameter, but in various lengths. A 30-cm tube was connected to the air valve and to a plastic cylinder (4 × 1 cm, length:diameter), which contained granular charcoal (Hummel Croton Inc., South Plainfield, New Jersey, USA). The charcoal was used to filter the air which came from the air valve and could absorb impurities which might be in the air flow. Both ends of the charcoal cylinder had a layer of fine mesh, so that the air could flow through it, but the charcoal particles could not pass into the rest of the system. A 15-cm tube connected the free end of the charcoal cylinder to a Y-shaped plastic connector. The rest of the system had two similar parts, 'a' and 'b'. For part 'a', a 30-cm tube connected one free end of the Y-shaped connector to a 15-cm glass tube (0.5 cm in diameter). A plastic stopper (2.5 cm tall and 3.3 cm diameter at top) which had two longitudinal holes, 0.5 cm in diameter each, was prepared. The glass tube was placed tightly into one of the holes, so that 10 cm of the glass tube protruded from the stopper. Another glass tube (4 × 0.5 cm, length:diameter) was inserted into the other hole of the

stopper; the small glass tube protruded 1 cm from each side of the stopper. The side of the small glass tube that protruded from the upper side of the stopper was attached to a 10-cm tube, and then attached to a Y-shaped connector. To each end of the Y-shaped connector, a 30-cm tube was attached. The stopper, which contained 2 glass tubes, was placed in an Erlenmeyer flask (Pyrex®, 250 ml, stopper No. 6, USA), hereafter called flask. Part 'b' was set up the same as part 'a'. All tubes and flasks were fixed on the Styrofoam with tape. For each of the parts 'a' and 'b', two 1-cm holes were made near the base of the wall of a clear plastic 250 ml container used to hold the spikes receiving the volatiles. The container was taped to the Styrofoam and the last two tubes of each part were inserted into the holes in the side of the container. The spaces around the holes were filled with cotton batting and taped. Two bamboo sticks (15 × 0.5 cm, height:diameter) were inserted vertically into the Styrofoam, at the centre of the container. The plastic tubes were taped vertically to the sticks, so that open ends of the tubes were 12 cm above the Styrofoam.

In part 'b', the test treatments were applied, while part 'a' was used as the control. In Test 1, the effect of volatiles of pre-anthesis 'Roblin' on oviposition rate on pre-anthesis 'Key 10' was explored. Twenty spikes of 'Roblin', at pre-anthesis stage (Zadoks growth stage 57-59: Zadoks *et al.* 1974), were excised from the peduncle. Six spikes of 'Key 10', at the pre-anthesis stage, were excised 15 cm below the flag leaf base. 'Roblin' and 'Key 10' spikes were transferred to the test room. The flasks were filled with 100 ml of distilled water. The flask of part 'a' was blocked with the stopper. The 'Roblin' spikes were inserted into the flask of part 'b' and the flask was blocked with the stopper. The bases of the spikes were oriented toward the base of the flask, so that they could absorb

water and stay alive during the test. The basal few spikelets were submerged in the water. In both flasks, the incoming air came through the longer glass tube into the flask, with the tip of the glass tube submerged in the water. When the air passed through the tube into the water, it made bubbles indicating that the system was working. Moreover, bubbling could increase the evaporation of water, which maintained a high relative humidity in the bottles needed for wheat midge oviposition. The six 'Key 10' spikes were cut to a height of 20 cm and placed into piks as described above. At the centre of each container, three 'Key 10' spikes were positioned, and the flag leaves were gently bent down. Two wheat midge-free emergence bottles were chosen and three female (24-48 h old) and two to three male wheat midges were released into each of the bottles. The bottles were transferred to the test room. The spikes were misted with water. It was ensured that the insects had landed on the upper part of the bottles before removing the base of the bottles. Next, the upper parts of the bottles which contained the wheat midges were inserted onto the containers which contained the plants and were taped to the Styrofoam. The bottles were taped to the base containers and the air valve was opened. After two nights the spikes were collected and dissected under a stereomicroscope. Before dissection, the length of the spike from base spikelet to the terminal spikelet was measured. Dissection started from the base spikelet and continued for each spikelet up the spike. Eggs laid on different parts of a spikelet were counted: eggs on the outer surface of the spikelet, eggs inside glumes (the outermost modified leaves of a spikelet), eggs inside and between florets, and eggs on the inter-spikelet part of the rachis together with the eggs laid on the surface of the spikelet facing the rachis. Test set-up and dismantling dates, number of wheat midges used, duration of test, name of the cultivar or line placed in the Erlenmeyer

flask, and name of the cultivar or the line placed in the bottles were also recorded. The distilled water of the Erlenmeyer flasks was renewed in each replication. Tests 2, 3, and 4 were similarly conducted. The differences among the tests were the source of the volatiles and the wheat receiving the volatiles, as described below.

Statistical analysis

Data were analyzed using procedures of SAS® (SAS Institute Inc. 2002). For the color experiment, each spike was considered as an experimental unit and the number of eggs per spike was analysed. A spikelet was not considered as an experimental unit, because when a wheat midge oviposits on a spikelet of a spike, she usually remains on the spike for a while and probes and oviposits on other spikelets of that spike (Chapter 5). For the chemical cues experiment, each spikelet was considered as an experimental unit for comparing the proportion of eggs laid on three parts of the spikelet, as it was assumed that the proportion of eggs laid on different parts of a spikelet was independent of the number of eggs laid on the different parts of the other spikelets. This was based on the fact that wheat midge oviposits on some of the spikelets of a spike, leaving the rest uninfested.

The normality of the untransformed and transformed data and also normality of residuals after analysis of variance were checked using stem-leaf and normal probability plots. Homoscedasticity was checked by observing graphical distribution plots of variance by mean (PROC PLOT). For the color experiment, data were square-root or log transformed to stabilize variance. If these transformations could not remove the heteroscedasticity, Taylor's Power Law was used to find the appropriate power for

transformation (Taylor 1961). A mixed model analysis of variance (PROC MIXED) was used to compare the egg numbers per spike for each treatment. Color was a fixed effect, and replication and spike were random effects. The Satterthwaite method was applied for determining the denominator degrees of freedom (d.f.). Comparisons among treatments were made using the Tukey-Kramer test where analysis of variance showed significant differences among means.

For the chemical cues experiment, numbers of eggs per female for treatments of each test were compared using a paired sample t-test (PROC TTEST) after checking the data for normality. The treatments of each test were considered paired, as they received the same air flow, wheat midges from the same culture and spikes which grew together under the same conditions.

A log linear model was used (PROC GENMOD: Poisson distribution, log link function) to test distribution of eggs on different parts of the spikelets of treatment and control of each test. A log linear model was repeated to test distribution of eggs on three sections (Upper, lower and middle one-third) of the spike of treatment and control of each test. In all experiments, differences between treatments were considered significant at $P < 0.05$ and mean values are given as the mean ± SE.

Results

Visual cues

The spectral analysis of the colors showed that the relative reflectance differed among colors (Figure 2.2). The red color folder had lower relative reflectance compared

to other colors at a wavelength range of 500-560 nm; the yellow color folder had a higher reflectance value in that range.

Test 1: four-color test

Assumptions of analysis of variance were met by a square-root transformation of data. There was a significant difference among the treatments (MIXED ANOVA: $F_{3,192}$ = 13.09, $P < 0.0001$). The highest egg densities were in the yellow treatment followed by blue, green and red (Table 2.1). The red treatment received significantly fewer eggs compared to the other treatments (Tukey-Kramer: $P < 0.0001$); however, differences among other treatments were not significant based on results of the Tukey-Kramer test ($0.2965 < P < 0.9334$) (Table 2.1).

Test 2: three-color test

Assumptions of analysis of variance were met by log transformation of data. There was a significant difference among the treatments (MIXED ANOVA: $F_{3,191} = 5.51$, $P < 0.0012$). The highest egg density was in the control treatment followed by yellow, blue and green (Table 2.1). Based on results of the Tukey-Kramer test, the green treatment had the lowest egg density compared with blue ($P < 0.0328$) and control ($P < 0.0006$) treatments, but was not significantly lower than the yellow treatment ($P < 0.0656$) (Table 2.1).

Test 3: black vs. control

Assumptions of analysis of variance were met by log transformation of data. There was a significant difference between the two treatments (MIXED ANOVA: $F_{1,123}$ = 6.27, P = 0.0107); the black treatment received fewer eggs (62 ± 7) than the control (92 ± 10).

Test 4: red vs. control

Assumptions of analysis of variance were met by transforming data using Taylor's Power Law (power for transformation = 0.28). There was a significant difference between the two treatments (MIXED ANOVA: $F_{1,85}$ = 37.74, $P < 0.0001$) and the red treatment received fewer eggs (84 ± 11) than the control (179 ± 16).

Chemical cues

Effect of wheat spike volatiles on number of eggs laid by wheat midge

The distributions of data were normal (Kolmogorov-Smirnov: $0.11 < D < 0.23$, $P > 0.1060$) for treatments of all tests (Table 2.2), justifying the use of the parametric paired sample t-test. In Test 1, no difference in egg density was detected between the control and treated spikes. The 'Roblin' spikes treated with post-anthesis 'Roblin' volatiles (Test 2) or pre-anthesis 'Key 10' volatiles (Test 3) received 43% and 39% fewer eggs compared with corresponding control 'Roblin' spikes, respectively (Table 2.2). In Test 4, the control 'Roblin' spikes treated with pre-anthesis 'Roblin' volatiles received 38% fewer eggs compared with control 'Roblin' spikes, but the difference in egg density on control and treated spikes was not significant (Figure 2.3; Table 2.2).

Effect of wheat spike volatiles on proportion of eggs laid on different parts of a wheat spikelet

The interaction effect shown in Table 2.3 explains if treatment volatiles had an effect on distribution of eggs on different parts of a wheat spikelet. In Test 1, in both control and treatment, most of the eggs were laid on the rachis compared to glume and floret (Figure 2.4; Table 2.3). In this test, the proportion of eggs laid inside the glume was lower for the treatment compared to the same part in the control (Figure 2.4). In Tests 2, 3 and 4, the proportion of eggs laid on the rachis was the lowest compared to the other parts, in both the control and treatment (Figure 2.4; Table 2.3). In Test 2, the proportion of eggs laid on different parts of the spikelet did not differ between control and treatment (Figure 2.4; Table 2.3). In Test 3, the proportion of eggs inside the floret of treated spikes was higher than for the control, and the proportion of eggs inside the glume of treated spikes was lower than for the control (Figure 2.4; Table 2.3). In Test 4, the proportion of eggs on the rachis of the treated spikes was higher than for the control, and the proportion of eggs inside the glume of treated spikes was lower than for the control (Figure 2.4; Table 2.3).

Effect of wheat spike volatiles on proportion of eggs laid on three sections of the wheat spike

The interaction effect shown in Table 2.4 explains if treatment volatiles had an effect on distribution of eggs on three sections of a wheat spike. In Test 1, in both the control and treated 'Key 10' spikes, the upper one-third of the spike received the highest proportion of eggs (Figure 2.5). The treatment had lower proportions of eggs on the lower

and middle one-third, and higher proportion on the upper one-third, compared to the same sections in the control (Table 2.4, Figure 2.5). In Test 2, the control 'Roblin' spikes received most of the eggs on their upper one-third section (Figure 2.5). The treatment received a lower proportion of eggs on the lower one-third, and higher proportion of eggs on the middle one-third, compared to the same sections in the control (Table 2.4, Figure 2.5). In the treatment, the proportion of eggs laid on the upper and middle sections did not differ. In Test 3 and test 4, proportion of eggs laid on three sections of the spike between control and treatment did not differ (Table 2.4, Figure 2.5).

Discussion

Background color was one of the visual cues which we intended to explore for its effect on oviposition of wheat midge in the laboratory. Color of the background affected oviposition rate of wheat midges on spikes of 'Roblin' spring wheat. Red and black background suppressed wheat midge oviposition on spring wheat spikes; however, yellow and blue did not. Field studies in the United Kingdom (Oakley and Smart 2002) showed that yellow traps were more attractive than blue ones for wheat midge. Studies on other cecidomyiids showed that host finding and acceptance were influenced by visual cues, such as color (Harris and Rose 1990; Sharma *et al.* 1990; Harris *et al.* 1993). Hessian flies laid more eggs on yellow, green and orange papers compared to blue and red papers (Harris and Rose 1990). Yellow, red and white were attractive for sorghum midge, *Contarinia sorghicola* Coq. (Diptera: Cecidomyiidae), while blue and black were the least attractive colors (Sharma *et al.* 1990). We suggest that cecidomyiids, such as wheat midge, respond to background color as a visual cue when approaching host plants,

and the background color might be important in the pre-landing phase of host acceptance behavior.

The low egg density on green in the four-color and three-color tests, along with the high egg density on the control in the three-color test, may indicate that wheat midge detects the contrast between the host spike and the background color. The contrast among objects was important in Hessian fly (Harris *et al.* 1993) and apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) (Owens and Prokopy 1986) when approaching host plants. The approaching behavior of Hessian fly was affected by the contrast between colors; with a black background, landing on white targets was more frequent than on black targets. Wheat midge may use the soil blackness and the contrast of spike greenness with sky to fly towards the top of the wheat plants to find spikes suitable for oviposition.

The practical use of the results of the visual cues experiment could be in studies of populations using color-traps (Lamb *et al.* 2002*b*; Oakley and Smart 2002), for which we recommend yellow. We are not sure if the color of the cages used in the screening tests in the laboratory might affect screening results and egg density of wheat midge. However, different color mesh used for screening sorghum lines against sorghum midge affected screening results (Sharma *et al.* 1990). Changing the color of wheat tissue is not possible without changing fundamental physiological processes, so the results can not be applied from that point of view.

Exploring the effect of chemical cues on oviposition of wheat midge was another objective of this study. Our hypothesis for Test 1 of the chemical cues experiment was: 'Roblin' produces pre-anthesis volatiles that stimulate oviposition; 'Key 10' is

antixenotic, and does not produce pre-anthesis stimulatory volatiles; adding stimulatory volatiles from 'Roblin' increases oviposition on 'Key 10'. The results did not support the hypothesis; adding pre-anthesis 'Roblin' volatiles did not affect oviposition rate on 'Key 10'. If pre-anthesis 'Roblin' had stimulatory volatiles, while 'Key 10' lacked the stimulatory volatiles, the pre-anthesis 'Roblin' volatiles would be expected to increase oviposition on 'Key 10'. In Test 3, the pre-anthesis 'Key 10' volatiles were added to pre-anthesis 'Roblin'. Our hypothesis for this test was: pre-anthesis 'Key 10' produces deterrent volatiles; adding deterrent volatiles of pre-anthesis 'Key 10' decreases oviposition on pre-anthesis 'Roblin'. Results did not reject the hypotheses. Pre-anthesis 'Key 10' volatiles significantly decreased oviposition on pre-anthesis 'Roblin'. This indicated that pre-anthesis 'Key 10' had deterrent volatiles which suppressed oviposition of wheat midge, and the antixenotic effect of 'Key 10' was not due to lack of stimulants. We intended to explore the effect of post-anthesis 'Roblin' volatiles on pre-anthesis 'Roblin' (Test 2). Previous research indicated that at the post-anthesis stage the oviposition of wheat midge decreased (Elliott and Mann 1996). Our hypothesis for Test 2 was: post-anthesis 'Roblin' produces deterrent volatiles; low egg density on post-anthesis 'Roblin' is not due to lack of stimulants; adding post-anthesis 'Roblin' volatiles can decrease oviposition on pre-anthesis 'Roblin'. Our results did not reject the hypothesis; post-anthesis 'Roblin' volatiles significantly decreased oviposition on pre-anthesis 'Roblin', indicating that post-anthesis 'Roblin' spikes produced deterrent volatiles. If low egg density on post-anthesis 'Roblin' was due to lack of stimulants, its volatiles could not decrease the oviposition on pre-anthesis 'Roblin'. There was no supportive data to conclude that the volatiles of pre-anthesis 'Roblin' were stimulatory. Test 4 of the

chemical experiment was conducted based on the hypothesis that pre-anthesis 'Roblin' has stimulants, and adding the stimulants can increase the oviposition rate on pre-anthesis 'Roblin'. Although the difference between control and treated spikes in Test 4 (Table 2.2) were similar to results of Tests 2 and 3, the difference was not statistically significant. We do not have a clear reason for this result. More research and repeating this test might clarify the problem.

This experiment provided strong evidence that wheat midge responded to volatiles of wheat spikes. In this study, the ratio of volatiles presumably was changed inside the bottles. The deterrent volatiles increased inside bottles, decreasing oviposition on pre-anthesis 'Roblin'. Birkett *et al*. (2004) also suggested that the wheat midge detected the host plants using a particular ratio of constitutive volatiles. The effect of deterrent post-anthesis volatiles of 'Roblin' explains partially the observed reduction in oviposition rate on post-anthesis 'Roblin' and pre-anthesis 'Key 10' in previous research (Lamb *et al.* 2003). Test 3 of the volatile experiment indicated a 39% reduction in oviposition rate on pre-anthesis 'Roblin' which was due to deterrent volatiles of the pre-anthesis spikes of 'Key 10'. Comparing this reduction to the 50% or more reduction which Lamb *et al.* (2002*a*) found, leads us to conclude that the main reason for antixenosis might be volatiles emitted from spikes. It is not known if the volatiles of post-anthesis spikes of 'Roblin' and pre-anthesis spikes of 'Key 10' are different compounds or the same compounds emitted at different growth stages. Several studies have shown the effect of plant age on production of chemical cues (Sutherland and Hillier 1974; Simpson and McQuilkin 1976; Visser 1976; Wallbank and Wheatley 1976). It is hypothesized that during the evolution of plant-insect interactions, wheat midge adapted

to the growth stage of the host plant and now uses volatiles to recognize when a host plant is at the post-anthesis stage and is no longer a suitable host.

The proportion of eggs laid on different parts of the spikelets of the test spikes might be affected by short range volatiles emitted by the test spikes and also by morphological traits of the spike. The proportion of eggs on the rachis of 'Key 10' was greater than on the glume and floret, which is corroborated by other research (Lamb *et al.* 2003). If this behavior was influenced only by volatiles, it was expected that in Test 3 the proportion of eggs on the rachis of the 'Roblin' spikes, which had received the 'Key 10' volatiles, would be higher than on the glume and floret. This was not the case. It is possible that tactile oviposition cues were detected by the ovipositor of the wheat midge. The length of hair inside the glume and lemma were longer in 'Key 10' compared with 'Roblin', and length of hair on the rachis of 'Roblin' was longer compared to 'Key 10' (Chapter 4). There is no information regarding the tactile receptors or chemoreceptors on or around the ovipositor of wheat midge or Hessian fly, but in a cecidomyiid, *Dasineura brassicae* Winn., tactile sensors were found around the oviduct (Hallberg and Åhman 1987). I hypothesize that the main factor in distribution of eggs on different parts of spikelets of 'Roblin' and 'Key 10' is tactile cues rather than chemical cues (Chapter 4).

Wheat midge laid the highest proportion of eggs on the upper one-third of the control spikes followed by the middle and lower one-third sections. However, in the treated spikes of Test 2 the pattern changed; the difference between upper and middle one-third sections was not significantly different. It is possible that as a wheat spike develops it produces compounds which are detected by wheat midge, affecting the distribution of eggs on the spike sections. Wheat spikes develop and flower in the middle

section first, followed by the upper and lower sections (Vries 1971). Higher oviposition rate on the middle and upper compared to the bottom section of the spike was observed previously (Barnes 1956; Oakley 1981; Kurppa 1989; Smith and Lamb 2001). However, in those studies the flag leaf had covered the bottom spikelets for part of the time, but in our study the flag leaf was rolled back and the whole spike was exposed to wheat midge. Therefore, the low egg density on the lower one-third section was due to the effect of chemical or tactile cues rather than being covered by the flag leaf. Detection of the kernels at the suitable growth stage by female wheat midges is important for larval survival, as the larvae can start feeding on newly developing kernels, and the kernels in other stages of development will not be suitable for the sedentary larvae.

In conclusion, wheat midge used visual and chemical cues in host finding and oviposition. The color contrast between wheat spikes and background was important for allocation of eggs on the wheat spikes. The red and black backgrounds were deterrent; however, yellow and blue backgrounds were not. Wheat midge detected volatiles produced by wheat spikes. Deterrent volatiles emitted by the post-anthesis 'Roblin' and pre-anthesis 'Key 10' decreased the oviposition rate on the pre-anthesis 'Roblin', and this suppression of oviposition was not due to lack of stimulants. Selection of suitable kernels by female wheat midges for the larvae was hypothesized to be affected by the volatiles of the wheat spike, and the distribution of eggs on a spikelet was hypothesized to be affected mostly by tactile cues rather than volatiles (Chapter 4).

More research is needed to scrutinize the interaction among visual and chemical cues and identify the specific compounds affecting oviposition behavior and deterrence. After identifying the compounds which confer deterrence, the compounds can be traced

in wheat lines with different genotypic backgrounds, which might clarify the mechanisms of resistance or susceptibility of host plants to the wheat midge. Synthetic deterrent compounds might be applied in the wheat field to reduce wheat midge oviposition (Bruce *et al*. 2002); however, their effect on natural enemies must be explored too. Field-grown spikes should be used to explore the effect of volatiles on egg density of wheat midge and compare the results with the laboratory-grown spikes, as there may be some interactions among the genes responsible for volatile production and environmental conditions (Jones *et al.* 1979; Donselman and Flint 1982; Larsson *et al.* 1986).

Table 2.1. Comparison of number of eggs per spike laid by *Sitodiplosis mosellana* on wheat spikes in front of different background colors in the laboratory. Means followed by the same letter do not differ significantly based on the Tukey-Kramer test.

Test	Replications	Mean±SE
Test 1	5	
Yellow		183±24 a
Blue		161±11 a
Green		139±8 a
Red		96±8 b
Test 2	5	
Control		140±16 a
Yellow		123±13 ab
Blue		114±10 a
Green		100±12 b

Table 2.2. Paired sample t-test comparing eggs per female *Sitodiplosis mosellana* (mean±SE) in different tests of the experiment exploring the effect of wheat spike volatiles on the oviposition rate of *Sitodiplosis mosellana*. Differences are significant at $P < 0.05$.

Test No.	Source of volatiles	Wheat receiving volatiles	Replications	No. of eggs / female		d.f.	*t*	*P*
				Control	Treatment			
1	Pre-anthesis 'Roblin'	Pre-anthesis 'Key 10'	15	33±6	31±4	14	0.35	0.7340
2	Post-anthesis 'Roblin'	Pre-anthesis 'Roblin'	13	42±5	24±5	12	2.84	0.0148
3	Pre-anthesis 'Key 10'	Pre-anthesis 'Roblin'	10	41±7	25±3	9	2.29	0.0481
4	Pre-anthesis 'Roblin'	Pre-anthesis 'Roblin'	10	42±7	26±5	9	1.69	0.1252

Table 2.3. Log linear analysis of distribution of wheat midge eggs on different parts of a spikelet of treatment and control wheat spikes in the volatile experiment. Each test had a 3×2 contingency table classified by spikelet part and treatment. Differences are significant at $P < 0.05$.

Test	Source of volatiles	Wheat receiving volatiles	Effect	d.f.	Chi-Square	*P*
1	Pre-anthesis 'Roblin'	Pre-anthesis 'Key 10'				
			Treatment	1	5.56	0.0184
			Spikelet part	2	757.05	<0.0001
			Treatment×Spikelet part	2	8.38	0.0152
2	Post-anthesis 'Roblin'	Pre-anthesis 'Roblin'				
			Treatment	1	171.55	<0.0001
			Spikelet part	2	361.05	<0.0001
			Treatment×Spikelet part	2	1.45	0.4845
3	Pre-anthesis 'Key 10'	Pre-anthesis 'Roblin'				
			Treatment	1	117.94	<0.0001
			Spikelet part	2	140.62	<0.0001
			Treatment×Spikelet part	2	21.28	<0.0001
4	Pre-anthesis 'Roblin'	Pre-anthesis 'Roblin'				
			Treatment	1	53.15	<0.0001
			Spikelet part	2	374.65	<0.0001
			Treatment×Spikelet part	2	27.23	<0.0001

Table 2.4. Log linear analysis of distribution of wheat midge eggs on three sections of a spike of treatment and control wheat spikes in the volatile experiment. Each test had a 3×2 contingency table classified by spike section and treatment. Differences are significant at $P < 0.05$.

Test	Source of volatiles	Wheat receiving volatiles	Effect	d.f.	Chi-Square	*P*
1	Pre-anthesis 'Roblin'	Pre-anthesis 'Key 10'				
			Treatment	1	0.02	0.8973
			Spike section	2	1181.15	<0.0001
			Treatment× Spike section	2	24.86	<0.0001
2	Post-anthesis 'Roblin'	Pre-anthesis 'Roblin'				
			Treatment	1	199.12	<0.0001
			Spike section	2	357.17	<0.0001
			Treatment× Spike section	2	23.15	<0.0001
3	Pre-anthesis 'Key 10'	Pre-anthesis 'Roblin'				
			Treatment	1	98.20	<0.0001
			Spike section	2	411.64	<0.0001
			Treatment× Spike section	2	4.68	0.0962
4	Pre-anthesis 'Roblin'	Pre-anthesis 'Roblin'				
			Treatment	1	92.44	<0.0001
			Spikelet section	2	390.74	<0.0001
			Treatment× Spike section	2	2.50	0.2862

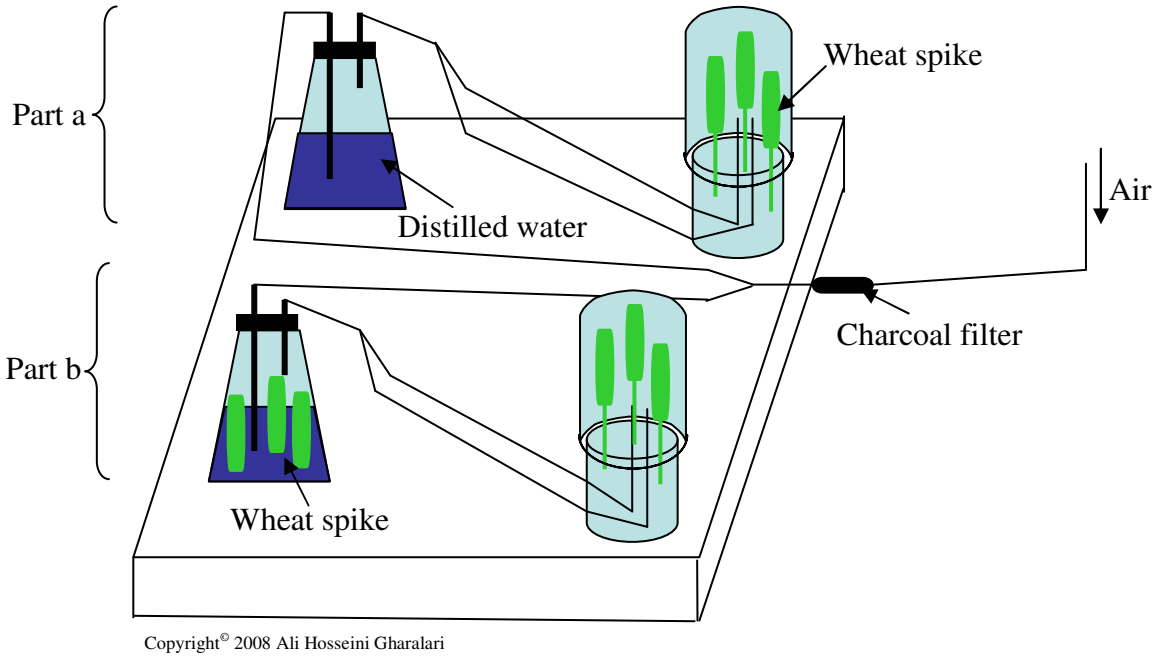


Figure 2.1. Schematic of the apparatus for exploring the effect of volatiles from bread wheat spikes on the oviposition rate of *Sitodiplosis mosellana* in the laboratory.

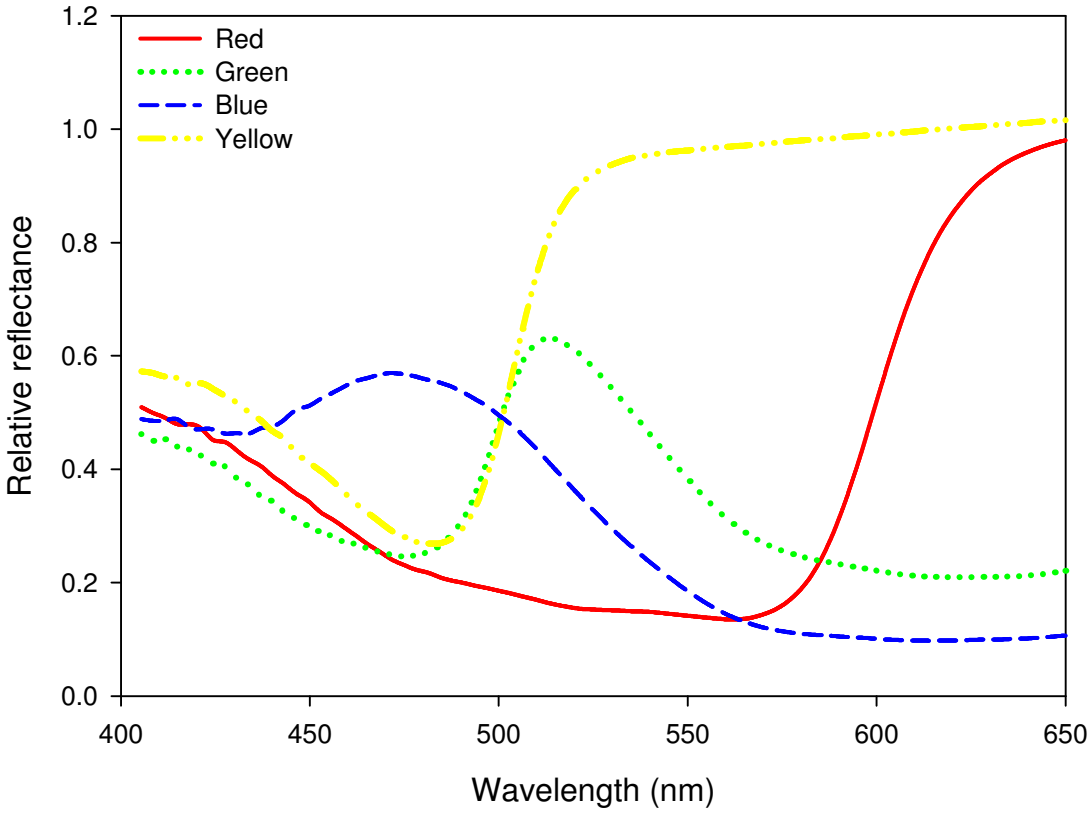


Figure 2.2. The relative spectral reflectance of the color folders used to explore the effect of background color of wheat spikes on oviposition rate of *Sitodiplosis mosellana* in the laboratory. The relative reflectance was measured relative to a white object (Teflon) reflectance spectrum.

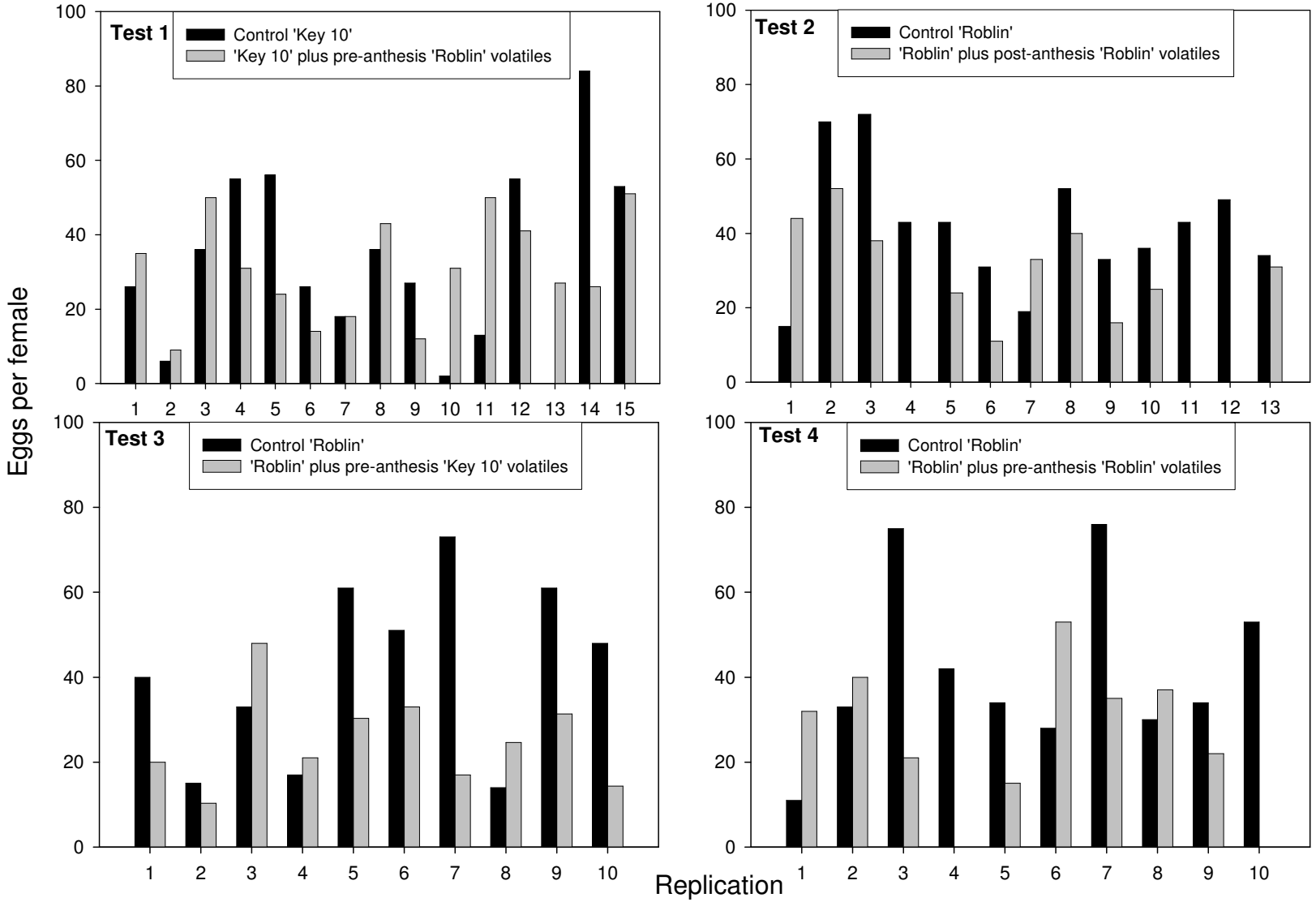


Figure 2.3. Effect of wheat spike volatiles on oviposition rate of *Sitodiplosis mosellana* on wheat in the laboratory.

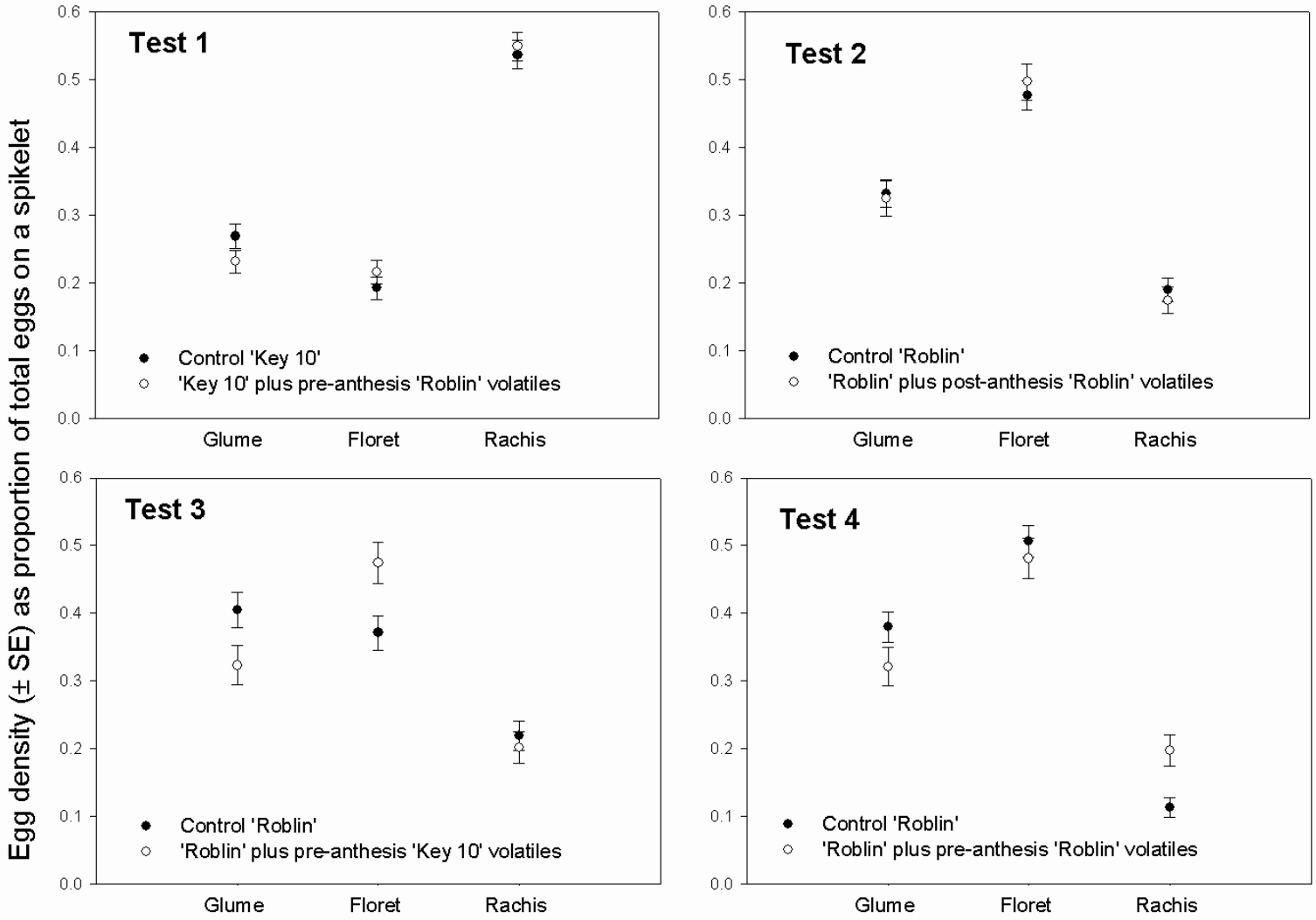


Figure 2.4. Effect of wheat spike volatiles on proportion of eggs laid by *Sitodiplosis mosellana* on different parts of the spikelets of wheat lines in the laboratory.

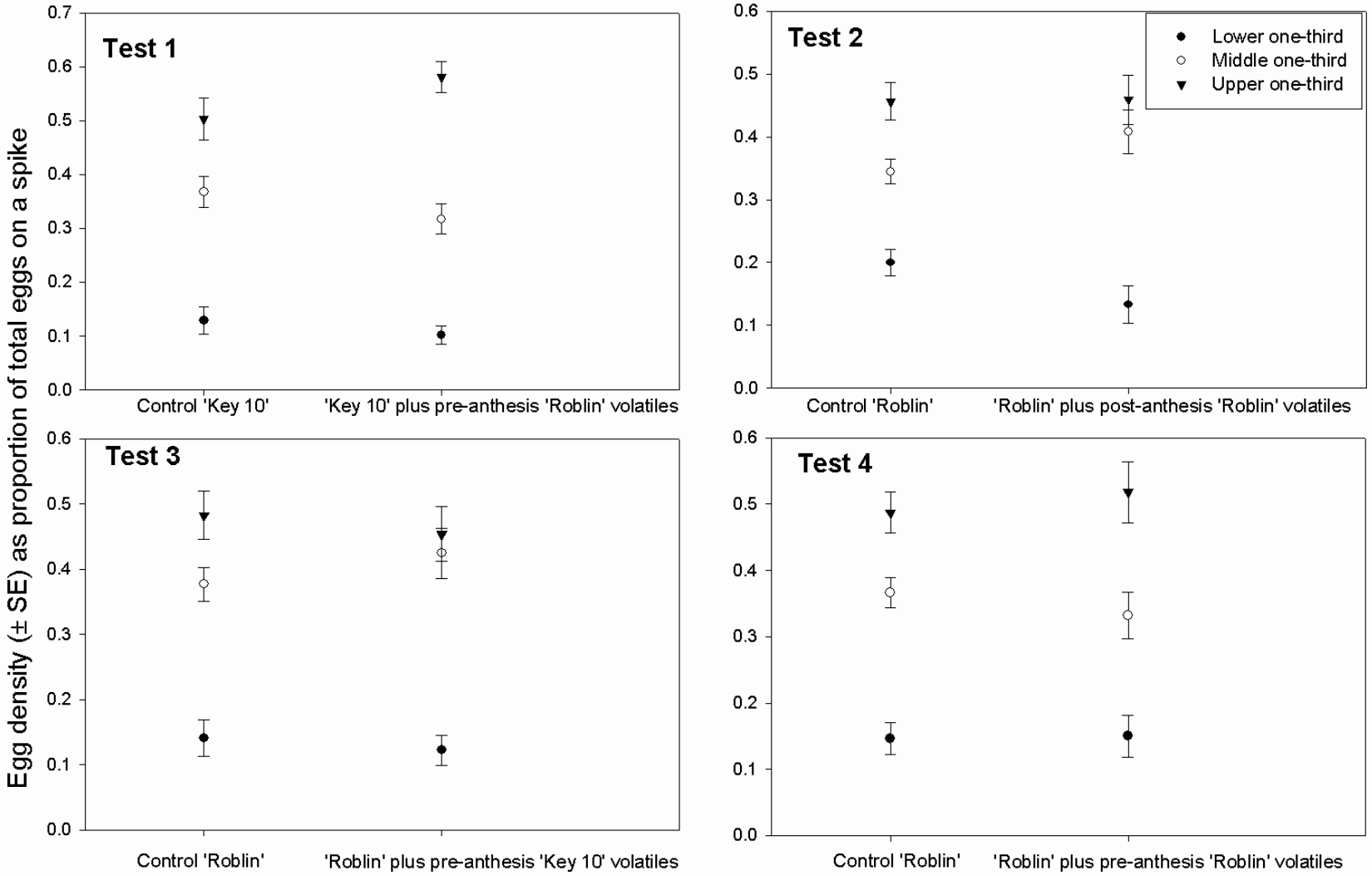


Figure 2.5. Effect of wheat spike volatiles on proportion of eggs laid by *Sitodiplosis mosellana* on three sections of wheat spike in the laboratory.

Chapter 3

Oviposition deterrence in spring wheat, *Triticum aestivum* L. (Poaceae), against wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), and implications for inheritance of deterrence

Introduction

Host-plant resistance (Snelling 1941; Painter 1951) has been found to be an economical, effective, and environmentally friendly approach for managing insect pest problems (Lindley 1831; Bioletti *et al.* 1921; Smith *et al.* 1999; Sharma and Oritz 2002). One of the resistance mechanisms in plants against pests is antixenosis (Kogan and Ortman 1978) which refers to the ability of a plant to reduce oviposition or feeding by an insect pest because the plant lacks a stimulant or possesses some deterrent and/or repellent traits, arising from chemical or morphological characteristics of the plant (Painter 1951). Antixenosis can be considered as the first barrier in a plant defence system against pests, as antixenosis decreases the number of colonisers on plants, for example, by decreasing the egg density on the plants.

Among cereals, wheat, *Triticum* spp. (Poaceae), might be considered as the most important crop (Johnson *et al.* 1978) because it can be grown across a wide range of environments, offers a good yield, is easily transported and stored, and is used to produce a large variety of products and foods (Briggle and Curtis 1987). Therefore, controlling wheat pests in agriculture has received special attention by researchers. Some of the major insect pests of wheat for which resistant varieties have been deployed include

wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae) (Kemp 1934; Stoa 1947; Cook *et al.* 2004) and Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae) (Dean and McColloch 1915; Painter *et al.* 1931). Virulent biotypes of Hessian fly have evolved (Painter 1930), which has made researchers look for diverse resistance sources and genes to control different biotypes of this pest. Since then, 31 antibiosis genes, symbolized as *H1* (Nobel and Suneson 1943) to *H32* (Sardesai *et al.* 2005), have been found in related plant species, mainly *Triticum* spp. (Gallun and Reitz 1971; Hatchet *et al.* 1981), *Secale* spp. (Hatchet *et al.* 1993), and *Aegilops* spp. (Delibes *et al.* 1997). These genes have been used in development of Hessian fly-resistant lines.

The wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), is a key pest of wheat in the northern Great Plains (Wright and Doane 1987; Lamb *et al.* 2000*a*). Females oviposit at dusk, from a short time before sunset until dark (Reeher 1945; Pivnick and Labbé 1993) on wheat spikes (Mukerji *et al.* 1988). Larvae feed on the surface of early-stage developing kernels (Ding and Lamb 1999), resulting in kernel shrivelling and reduction in quality and quantity of yield (Miller and Halton 1961; Olfert *et al.* 1985). In addition to the widespread application of insecticide to control this pest (Elliott 1988*a*, 1988*b*), resistant cultivars to the wheat midge (Barker and McKenzie 1996; Ding *et al.* 2000) containing the antibiosis gene *Sm1* (McKenzie *et al*. 2002) have been developed. The antibiosis is associated with high constitutive and induced levels of phenolic acids (Ding *et al.* 2000). Because antibiosis is conferred by a single gene, which confers high pressure on wheat midge population, it is likely to break down rapidly (Smith *et al.* 2007); therefore, other resistant lines with new resistance genes or additional resistance mechanisms such as antixenosis are needed. Theoretically, pyramiding

resistance genes can delay the evolution of virulent biotypes of pests (Gould 1986). Research at the Cereal Research Centre, Agriculture and Agri-Food Canada, resulted in production of antixenotic wheat lines against wheat midge, which decrease wheat midge egg density on resistant wheat lines (Ding and Lamb 1999; Smith and Lamb 2001; Wise *et al.* 2001; Lamb *et al.* 2002*a*). It is suggested that more than one gene controls antixenosis (Lamb *et al.* 2002*a*).

This study uses the resistance sources discussed above to explore the genetic basis of antixenosis. The objectives were: to study antixenosis inheritance including exploration of the number of antixenosis genes and type of interaction among these genes; to determine linkage, if any, between the antibiosis gene and antixenosis genes; to determine linkage, if any, between genes suppressing awn production and antixenosis genes; to explore the association between antixenosis and spike length and compactness, and to determine if antixenosis affects distribution of eggs on spikes and spikelets.

Materials and Methods

Laboratory screening of doubled-haploid spring bread wheat lines for antixenosis to wheat midge

Ninety-two doubled-haploid spring wheat lines and their parents, 'Roblin' and 'Key 10' (Appendix 1), were screened in the laboratory for antixenosis to wheat midge. 'Roblin' is a wheat midge-susceptible cultivar (Lamb *et al.* 2000*a*) with high yield and good agronomic traits. 'Key 10' has antibiosis and antixenosis to wheat midge (Lamb *et al.* 2000*a*). Ten spikes of each line were screened, but just 32 spikes could be placed in each cage, and spike dissection was time consuming; therefore, the lines were screened

over a period of several months. Due to variation among cages in environmental conditions (temperature, humidity, light intensity), in growth conditions of plants, and wheat midge physiological status, lines screened in each cage included the control lines, 'Roblin' and 'Key 10'. Therefore, the results for the lines tested in each cage could be compared to the results observed for the control lines in that cage.

The general seeding procedure and plant culture in the laboratory was the same as described in Chapter 1. Each week, 4 or 5 plants of each of 10 lines were seeded, as well as 25 plants each of 'Roblin' and 'Key 10'. 'Key 10' headed about 4 to 5 days later than 'Roblin', so in the first tests, just 'Roblin' was used as a control. Lines that did not germinate the first time were seeded again.

Out of 32 spikes in each cage, two were 'Roblin', two were 'Key 10' and the rest were the doubled-haploid lines which were to be tested. At the heading stage, when 75% to 100% of the spike had come out of the flag leaf (Zadoks growth stage 57-59: Zadoks *et al.* 1974), at least two spikes per line were excised; all leaves except the flag leaf were cut off, the spikes were labelled with the code numbers of the lines, and transferred to the test room.

In the test room, a 60 × 60 × 5 cm Styrofoam block was positioned on the base frame of the cage. The upper surface of the block had a six-by-six array of 5 mm holes, spaced 5 cm from each other. The edges of the array were 15 cm from the edges of the cage. The holes were numbered in clockwise helix form, starting from the left upper hole. Out of 36 holes, the four holes at the centre of the array were not used because previous research indicated that wheat midge did not lay many eggs on spikes located in these positions (Smith and Lamb 2001). Before starting a test, the position of each spike in the

32-hole array was randomized using SAS® software (SAS institute Inc. 2002). The code numbers of lines and their corresponding positions were written on the piks with a permanent marker. The spikes were inserted into the piks as described in Chapter 2 and positioned into the numbered holes. If the flag leaf covered some spikelets, it was folded back gently exposing the whole spike. The spikes and inside of the cage were misted thoroughly with water until droplets appeared on the inner surface of the cage walls. The cage was positioned on the Styrofoam block and taped in place. Midges were released into each cage at a rate of one female and one male midge per spike. After three nights, the cage was dismantled and the piks containing the spikes were transferred to the laboratory for dissection. The cage set-up date, dismantling date, and code numbers of lines used were recorded.

Before dissecting each spike under a stereomicroscope, the spike length in centimetres from the base of the first spikelet to the tip of the terminal spikelet, the number of spikelets, and whether it was awned or awnless were recorded. Awns were trimmed to ease the dissection process; females do not lay eggs on awns. Data recorded for each spikelet included: a) number of eggs on the outer surface of the spikelet, b) number of eggs inside glumes (the outermost modified leaves of a spikelet), c) number of eggs inside and between florets, and d) number of eggs on the inter-spikelet part of the rachis together with the eggs laid on the surface of the spikelet facing the rachis. The process of setting up cages was repeated until 10 spikes per line were screened.

Field screening of doubled-haploid wheat lines for antixenosis and antibiosis to wheat midge

In addition to the laboratory screening, the 92 doubled-haploid wheat lines and their parents were screened in the field. The field tests were conducted during the summers of 2006 and 2007 at Brandon, Manitoba, Canada (49° 79′ N, 100° 02′ W). This location was selected because Brandon was expected to have high wheat midge pressure. Screening was also attempted at Glenlea, Manitoba, Canada (49° 38′ N, 97° 7′ W), but was not successful due to unsuitable weather conditions and very low wheat midge pressure.

At Brandon, there were three seeding dates in 2006 (12, 19 and 25 May), and three seeding dates in 2007 (15 and 28 May, and 3 June). Several seeding dates ensured that plants seeded on at least one of the dates would encounter peak densities of ovipositing midges. The lines were expected to head at different times, but they had to be screened on the same dates while all the lines were at the same level of midge pressure, so spikes to be screened were selected from the seeding date where they were at the required growth stage.

The positions of lines in the field plots were randomized using SAS® (SAS Institute Inc. 2002). The seeds of each line were seeded in a 1-m row (Figure 3.1) using a motorized belt seeder. The outermost rows were seeded with winter wheat. The rows adjacent to the outermost rows were seeded with control lines, ‘Key 10’ and ‘Roblin’, which were seeded alternately along the sub-blocks (Figure 3.1).

The procedure for sampling spikes in the field was similar for 2006 and 2007. To monitor for the presence of ovipositing female wheat midges, 10 spikes of ‘Roblin’ were

collected in early July, transferred to the laboratory and dissected to find wheat midge eggs. When ovipositing females were known to be present, 40 spikes of each line which were 50% to 100% out of flag leaf (Zadoks growth stage 54-59: Zadoks *et al.* 1974), were tagged using small pieces of red tape around the culm, below the flag leaf. For each parent line, 20 spikes were tagged in total throughout all control rows. The tagging date in 2006 was 13 July, and in 2007 was 9 July. Tagging spikes to be sampled ensured that they were all at the same stage and under the same level of wheat midge pressure, so the lines could be compared with each other.

After 4 nights, 20 tagged spikes of each line were collected for egg counts. In 2006, the samples of each line were bundled into 2 groups of 10 with a tape, labelled with the code number of each line and placed in paper bags. In 2007, the plant samples of each line were placed in 2 labelled coin envelopes (size #3), each containing 10 spikes, and placed in plastic containers with snap-on lids. In each year, samples were transferred to a 2.5°C cold storage room.

Two weeks after the date of egg sampling, 27 July in 2006 and 23 July in 2007, 10 tagged spikes per line were sampled for larval counts, to determine antibiosis levels. The samples were collected as described for egg-count samples and stored in a 2.5°C cold storage room until they were dissected.

The dissection procedure for egg counts was the same in 2006 and 2007. Samples were taken out of the cold storage room a few at a time and were put in Ziploc plastic bags to keep them fresh until dissected. The dissection procedure and the data that were recorded for each line were the same as described above for the laboratory screening. However, one bundle or bag of 10 spikes of each line was dissected first; then, the

remaining 10 spikes were dissected. This ensured that at least half of the samples for all lines could be dissected in the shortest time possible, because, even by keeping the samples at low temperature, some eggs hatched. This resulted in some loss of data, because within-spikelet locations of eggs from which larvae hatched could not be recorded. Number of larvae was recorded, so that the total number of eggs in each spikelet could be calculated.

The spikes for larval counts were dissected under a stereomicroscope. The spike length (in centimetres from base to terminal spikelet) and number of spikelets were recorded for each spike before dissection. Dissection began from the spikelet located at the base of the spike, and the number of dead larvae, second instars, third instars, and larval exuviae were recorded for each spikelet. There were no first instars present to be counted. Presence of larval exuviae indicated that the third instars successfully completed development and came out of the spikelet. Once third instar larvae, larval exuviae or dead larvae were found, dissection was stopped, because the presence or absence of antibiosis could be assessed.

Statistical analysis

Data were analyzed using procedures of SAS® (SAS Institute Inc. 2002). Where needed, square-root or log transformation was applied to remove heteroscedasticity and normalize distributions of data, so the assumptions of analysis of variance would be met. If the above transformations were not effective, Taylor's Power Law (Taylor 1961) was applied to find the appropriate power for data transformation. The normality of variables was checked using stem-leaf and normal probability plots (PROC UNIVARIATE).

For field-screening data, a mixed model analysis of variance (PROC MIXED) was applied to compare the number of eggs per spike on test lines with the control lines. Line was a fixed effect; spike was a random effect. The Dunnett test was applied to compare each line with each of the control lines separately. Based on the results of the Dunnett test and mean number of eggs per spike, the lines were grouped into different antixenosis levels (Table 3.1). Data of each year were analyzed separately.

For laboratory-screening data, a mixed model analysis of variance (PROC MIXED) was applied to compare the number of eggs per spike on each test line in each cage with the control lines of the same cage. Line was a fixed effect. Line × cage, cage and spike were considered as random effects. The analysis of variance was repeated separately for each of the test lines. Two analyses were done for each test line, comparing it with each control line separately. Therefore, the fixed effect included just a test line and a control line. Based on the results of the analysis of variance and mean number of eggs per spike, the lines were grouped into different antixenosis levels, as was done above for field data (Table 3.1).

A second method (see Figure 3.2) was used to categorize lines into two categories: 1) 'CR': the lines which were consistently resistant in the laboratory and two field seasons; and 2) 'NR': the remaining lines which did not belong to category 1. The rest of the analysis was done on the two categories.

For the field and laboratory screening tests, mean egg density on the test lines as a proportion of number of eggs on 'Roblin' was calculated. Correlations (r_p) between field and laboratory proportions were estimated (PROC CORR). The same data were entered into GGEbiplot software (Yan 2001) and analyzed based on the Singular Value

Decomposition method, to visually explore the relationship among environments and genotypes. GGEbiplot analyzes the data based on Principal Component Analysis, and outputs the results as an interactive image. GGEbiplot can rank the genotypes and environments and show their relationship in a multi-environment experiment.

Before analysing the data for proportion of eggs laid on different parts of the spikelets, the spikelets were sorted based on egg hatch percentage. In 2006 field data, 5 spikelets out of 20871 had egg hatch range of 7% to 14%. The frequency of spikelets with some eggs hatched was high (e.g. 8 or more) at egg hatch rates of 16% or more. So, for 2006 field data the spikelets with 16% or higher egg hatch rate were excluded, which amounted to 619 spikelets out of 20871. To be consistent for field data analyses, the 16% level was applied for 2007 field data. For 2007 field data, 350 spikelets out of 25050 were excluded.

A log linear model was used (PROC GENMOD: Poisson distribution, log link function) to test distribution of eggs on different parts of the spikelets of the two antixenosis categories. A log linear model was repeated to test distribution of eggs on three sections of the spike of the two antixenosis categories.

In order to explore the differences in the proportion of awnless lines between the two antixenosis categories, a logistic regression modelling (PROC GENMOD: binomial distribution, logit link function) was applied, with awn as a dependent and antixenosis categories as an independent variable.

In order to test the relationship between antibiosis and antixenosis levels in the field, the number of antibiotic lines of the two antixenotic categories were analysed by logistic regression model (PROC GENMOD: binomial distribution, logit link function),

with the antibiosis level (0 = *Sm1* gene absent; 1 = *Sm1* gene present) as dependent effect and antixenosis level as fixed effect.

In order to test the relationship between antibiosis in the field and presence of awns, the proportions of awned and awnless antibiotic lines were analysed by logistic regression model (PROC GENMOD: binomial distribution, logit link function), with the antibiosis level (0 or 1) as dependent effect and awn level as fixed effect.

For the laboratory and field data, spike length (from base to the terminal spikelet in centimetres) of the lines was analyzed using a mixed model analysis of variance (PROC MIXED). Line was a fixed effect, and spike was a random effect. The analysis was repeated to compare the spike length between antixenosis levels (PROC MIXED), with antixenosis level as a fixed effect, and spike and line (nested within resistant level) as random effects.

Spike compactness was calculated as the number of spikelets in a spike divided by the corresponding spike length (from base to the terminal spikelet in centimetres). For the laboratory and field data, spike compactness of the lines was analyzed using a mixed model analysis of variance (PROC MIXED). Line was a fixed effect, and spike was a random effect. The analysis was repeated to compare spike compactness between antixenosis levels (PROC MIXED), with antixenosis level as a fixed effect, and spike and line (nested within resistant level) as random effects.

For estimating the broad sense heritability (h_b^2) of antixenosis, the egg density of the field data were subjected to analysis of variance (PROC GLM), with line × year, line and year as random effects. The variance due to the lines was considered as genotypic variance (σ_g^2), which was divided by phenotypic variance (σ_p^2) to obtain the broad sense

heritability. The phenotypic variance was estimated as (σ_g^2) + (σ_{gy}^2 / year) + (σ_{error}^2 / year × sample size), where σ_{gy}^2 is variance of the genotype × year interaction.

Skewness and kurtosis were used to detect gene interaction in the doubled-haploid population. The skewness of a biparental doubled-haploid population is equal to zero in the absence of gene interaction; it is greater in the presence of complementary gene interaction and smaller than zero when duplicate gene interaction is present. The kurtosis is negative or zero in the absence of gene interaction. It is positive in the presence of gene interaction (Choo and Reinbergs, 1982*b*). Fisher (1950) has given relations for estimating variance (k_2), skewness (k_3) and kurtosis (k_4) from samples of a population. The statistical tests of significance of k_3 and k_4 can be performed by calculating the coefficient of skewness ($g_1=k_3k_2^{-3/2}$), and the coefficient of kurtosis ($g_2=k_4k_2^{-2}$), and then comparing the two coefficients with their respective sampling standard errors. The sampling errors (SE) of g_1 and g_2 have been given by Fisher (1950).

Treatments were considered significantly different at $P < 0.05$, and the mean values are presented as mean ± SE.

Results

The number of analyzed lines was less than 94 in some screening tests (Laboratory: 90 lines; Field 2006: 85 lines; Field 2007: 90 lines), because some of the lines did not germinate at all or germinated sooner or later than the time needed for the test. Moreover there was a seed mix-up in the field test and so the affected lines were excluded. For the 2006 field-screening data, assumptions of analysis of variance of the number of eggs per spike were met by log transformation of data. For 2007 field data,

assumptions of the above analysis were met using Taylor's Power Law (power for transformation = 0.31). The egg density in the field differed significantly among lines in 2006 (MIXED ANOVA: $F_{84,1446} = 4.99$, $P < 0.0001$) and 2007 (MIXED ANOVA: $F_{89,1623} = 14.17$, $P < 0.0001$). Based on the results of the Dunnett test (Appendix 2.a), the lines were categorized (Table 3.1) into susceptible (47 lines), resistant (32 lines) and highly resistant (6 lines) levels in 2006; and susceptible (3 lines), moderately resistant (38 lines), resistant (48 lines) and highly resistant (1 line) in 2007.

For the laboratory-screening data, the stem-leaf and normal probability plots showed that the distribution of eggs per female was normal for the pooled data. In one of the cages out of a total of 41, egg density on 'Roblin' was 39 ± 20 and on 'Key 10' was 34 ± 22; this cage was excluded from the analysis. In the rest of the cages, 'Key 10' received ≤ 60% as many eggs as 'Roblin'. Data were transformed using Taylor's Power Law (power for transformation = 0.31), so assumptions of analysis of variance were met. Based on the analysis of variance results (Appendix 2.b), the lines were categorized (Table 3.1) into highly susceptible (2 lines), susceptible (19 lines), moderately resistant (17 lines), resistant (49 lines) and highly resistant (3 lines) levels.

In order to plot the egg densities in 2006 versus egg densities in 2007, the egg densities on test lines were standardized as the proportion of egg density on 'Roblin' (Figure 3.2). Resistance categories for the 2006 and 2007 field trials are shown on the y and x axes, respectively, and the categories for the laboratory trials are represented by letters for the five observed categories. Some lines were consistently resistant and some lines consistently susceptible in the screening tests. There were 25 test lines which were consistently resistant or highly resistant in the field and laboratory tests (Figure 3.2).

These lines are identified by the symbol 'CR' in the analyses below. There were two lines which were susceptible in the field and laboratory tests.

The GGEbiplot showed that 'Roblin' was susceptible and 'Key 10' was resistant in all environments (Figure 3.3.a). The GGEbiplot indicated that the laboratory screening and 2007 field-screening data were more similar to each other compared to 2006 field-screening data (Figure 3.3.b), because their vectors are close to each other. The first two principal components accounted for 93.7% of the total variation. The correlation coefficients of standardized egg density between laboratory and 2006 field (Table 3.2) were half of correlation coefficients of standardized egg density between laboratory and 2007 field. The correlation coefficient of standardized egg density on lines between 2006 and 2007 field test (Table 3.2) reflected the genotype × environment interaction (r_s = 0.406).

The proportions of eggs laid on exposed surfaces of the spikelets were close to zero in all antixenosis levels of laboratory and field tests, so they were not shown in Figure 3.4. This observation was consistent among laboratory and field tests. In the laboratory and field tests, the proportion of eggs laid on the rachis increased and proportion of eggs laid inside the floret decreased with increase in antixenosis level from susceptible to resistant (Figure 3.4; Table 3.3). The interaction effect shown in Table 3.3 explains the results.

In all tests, the lower one-third section of the spikes received the lowest egg density compared to the middle and upper one-third sections (Figure 3.5). In the field tests, the upper one-third section of lines in both antixenotic categories received the highest proportion of eggs. In the laboratory, the proportion of eggs laid on the upper

one-third decreased and the proportion of eggs laid on the middle one-third increased in the lines categorized as consistently resistant compared to the lines of the other category (Figure 3.5; Table 3.4). The interaction effect shown in Table 3.4 explains the results.

To compare the egg density of the awned and awnless lines in 2006 in the field, the assumptions of analysis of variance were met by log transformation of data. The egg density did not differ between awned (6 ± 1) and awnless (8 ± 1) lines (MIXED ANOVA: $F_{1,79.5} = 2.55$, $P = 0.1140$) in 2006 field data. For the 2007 field-screening test, Taylor's Power Law was used for data transformation (power for transformation = 0.31). The egg density on awnless lines was higher (58 ± 1) compared to awned lines (41 ± 1) (MIXED ANOVA: $F_{1,88} = 0.0011$, $P = 0.0011$) in 2007 field data.

Frequency distribution of awnedness was not correlated with antixenosis levels (Logistic regression: $\chi_1^2 = 0.17$, $P = 0.6803$) (Awned CR: 15 – Awnless CR: 10; Awned NR: 30 – Awnless NR: 37). There was no relationship between antibiosis levels and antixenosis levels (Logistic regression: $\chi_1^2 = 2.29$, $P = 0.1305$) (Frequency distribution: Antibiotic CR: 8 – Non-antibiotic CR: 17; Antibiotic NR: 27 – Non-antibiotic NR: 27).

For analysis of spike length of lines in the laboratory and field tests, assumptions of analysis of variance were met by log transformation of data. The spike length (in centimetres) differed significantly among lines in 2006 (MIXED ANOVA: $F_{84,1463} = 19.14$, $P < 0.0001$), 2007 field (MIXED ANOVA: $F_{89,1623} = 16.62$, $P < 0.0001$) and laboratory tests (MIXED ANOVA: $F_{89,241} = 4.97$, $P < 0.0001$). The spike length did not differ significantly between antixenosis levels in 2006 (MIXED ANOVA: $F_{1,82.8} = 0.97$, $P = 0.3268$), 2007 field (MIXED ANOVA: $F_{1,88.4} = 0.63$, $P = 0.4304$) and laboratory tests (MIXED ANOVA: $F_{1,92.7} = 0.05$, $P = 0.8265$).

For analysis of spike compactness of lines in the laboratory and field tests, assumptions of analysis of variance were met by log transformation of data. The spike compactness differed significantly among lines in 2006 (MIXED ANOVA: $F_{84,1446}$ = 19.97, $P < 0.0001$), 2007 field (MIXED ANOVA: $F_{89,1623} = 20.25$, $P < 0.0001$) and laboratory tests (MIXED ANOVA: $F_{89,1106} = 8.85$, $P < 0.0001$). The spike compactness in 2006 field was higher in non-antixenotic line (1.68 ± 0.005) compared to antixenotic lines (1.60 ± 0.008) (MIXED ANOVA: $F_{1,83.3}$ =7.00, $P = 0.0097$). In 2007 field (MIXED ANOVA: $F_{1,88.1} = 2.39$, $P = 0.1257$) and laboratory tests (MIXED ANOVA: $F_{1,87.8}$ = 1.89, $P = 0.1729$), spike compactness was not different between antixenosis categories.

The analysis of variance of log transformed data of egg density on the wheat lines in the field (Table 3.5) showed that the genotypic variance (σ_g^2) was 0.12318. The phenotypic variance (σ_p^2) was 0.18313 ($\sigma_p^2 = (0.12318) + (0.09338 / 2) + (0.53056 / 2 \times 20)$). The broad sense heritability h_b^2 was estimated to be 67%. The skewness and kurtosis were both positive for the field data (Table 3.6).

Discussion

This study revealed considerable variation among the spring bread wheat lines screened for egg density of wheat midge in the laboratory and over two successive years at field sites at Brandon, Manitoba. The screened doubled-haploid lines were homozygous and eased the classification of the antixenotic phenotype of a line. Antixenosis was effective at reducing oviposition in the laboratory and field. The least preferred line received 7% as many eggs as on ‘Roblin’ in the laboratory, 13% in the field in 2006, and 11% in the 2007 field test. The least preferred lines were not the same lines

in either of the field tests or laboratory test. There were 25 lines out of 92 which were consistently antixenotic against wheat midge. No line was found to escape oviposition completely, indicating that the lines had the minimum requirement for oviposition by wheat midge. However the antixenotic factors, possibly deterrent volatiles produced by the spike (Chapter 2), significantly suppress oviposition by wheat midge. The significant differences in antixenosis levels in the field indicated that the observed results of the laboratory screening test were not an artifact of the laboratory environment. The pattern of egg allocation among different parts of the spikelet and three sections of the spike was different between antixenosis levels, and possible implications of the observed patterns are discussed. The implications of this study for genetics of antixenosis are also discussed.

The proportion of eggs laid on different parts of the spikelet (glume, floret, rachis and exposed surface) of the tested wheat lines differed between antixenosis levels. The important and consistent pattern was the increase in proportion of eggs on the rachis and decrease in proportion of eggs inside the floret as antixenosis increased from susceptible to resistant. There might be some morphological and/or chemical factors causing this phenomenon. Finding the reasons for this egg allocation pattern may be helpful in detecting antixenosis genes. For the chemical factors, the experiment on the wheat spike volatiles (Chapter 2) showed that volatiles of a susceptible bread wheat cultivar, 'Roblin', at pre-anthesis stage does not decrease the proportion of eggs laid on the rachis of the pre-anthesis antixenotic line, 'Key 10'. Also, the volatiles of the pre-anthesis 'Key 10' did not increase the proportion of eggs on the rachis of the pre-anthesis 'Roblin' to higher levels than the proportion of eggs inside glume and floret of the pre-anthesis 'Roblin'.

For the morphological factors, the measurement of the morphological traits of the spikes of the tested lines (Chapter 4) showed that the hairs inside glume and lemma were longer in 'Key 10' compared to 'Roblin', and in 'Roblin' the length of hair at the rachis edge was longer compared to 'Key 10'. Therefore, it was hypothesized that in the parent wheats, 'Roblin' and 'Key 10', the hairs at different parts of the spikelet affected the egg allocation pattern. However, the reasons for egg allocation patterns in the progeny of the 'Roblin' × 'Key 10' cross is not clear. It might be due to some interaction between deterrent volatiles and unknown factors related to antixenosis or oviposition behavior. More research is needed to clarify this pattern observed in this and other studies (Chapter 2; Lamb *et al.* 2003).

The proportion of eggs laid on three sections of the spike (upper, middle and lower one-third sections) differed between antixenosis levels in the laboratory and field tests. The proportion of eggs on the lower section of the spike was always the lowest compared to the other two sections. In the field, the upper section received the highest proportion of eggs in the susceptible and resistant lines; however, in the laboratory tests, from susceptible to resistant level, the proportion of eggs on the upper one-third section decreased, and the proportion of eggs on the middle one-third section increased. In the laboratory, the flag leaf of each line was rolled back before a screening test so all the spikelets of a spike were exposed to wheat midge. Also, in the laboratory screening test, we could easily choose spikes which were in a similar growth stage, while in the field it was more difficult. It is hypothesized that as wheat develops, the spike emits some volatiles or develops some tactile cues which affect the oviposition pattern of wheat midge on the three sections of the spike. Wheat spikes develop and flower in the middle

section first, followed by upper and lower sections (Vries 1971). Higher oviposition rate on middle and upper spikelets compared to bottom spikelets was observed previously (Barnes 1956; Oakley 1981; Kurppa 1989; Smith and Lamb 2001). The pattern of egg distribution in spikes in the laboratory differed from the field, and it is suggested that pattern of the development of the spike of the resistant lines might be affected by environmental conditions or the genes which produce the volatiles, while wheat growth might be affected by the environment, resulting in changes in oviposition pattern on resistant wheat lines. More research is needed to explore these factors.

'Key 10' was the antixenotic and antibiotic awned parent of the doubled-haploid population. It was hypothesized that genes conferring antixenosis might be linked to the gene, *Sm1,* which confers antibiosis. There might be a linkage between genes suppressing awn and antixenosis. The linkages might simplify the production of lines which are resistant to wheat midge, because the DNA marker for *Sm1*, which is on chromosome 2B, is available (Thomas *et al.* 2001) and the genetic map of the genes suppressing the production of awns is also known (Slinkard 1998). However, our study did not support these hypotheses. There could be a linkage between *Sm1* and genes conferring antixenosis, but the fact that there is more than one gene conferring antixenosis makes it difficult to detect the linkage.

In the laboratory, each screening cage test was run for three nights, and the midge pressure was one female and one male wheat midge per spike in each cage. It is assumed that this midge oviposition pressure was high enough to discriminate between the lines based on antixenosis level. It is suggested that high wheat midge pressure and suitable environmental conditions in the 2007 field test were two of the reasons for good

discrimination among the lines regarding antixenosis level. This resulted in a similarity between the laboratory and 2007 field screening tests as the GGEbiplot showed. The lower similarity of the laboratory and 2006 field test might have been due to low wheat midge pressure and less suitable environmental conditions for wheat midge oviposition in the field. As GGEbiplot analysis showed, overall, 'Roblin' was susceptible and 'Key 10' was resistant. It was necessary to categorize the progeny lines into different antixenosis levels. The lines that were highly susceptible or highly resistant in one environment were not in the same category in other environments. This might be due to the effect of environment and field status on midge behavior which probably resulted in escape of some lines from heavy infestation by wheat midge eggs. However, the 25 lines which were consistently resistant in all environments can be a good source for further research and might be used in future breeding programs.

In this study, there were some lines which were more deterrent than the antixenotic parent, 'Key 10'. If all antixenosis genes had originated from the resistant parent, it was expected that the level of the antixenosis of the progeny would be between the antixenosis level of the two parents. However, the presence of progeny lines which were more antixenotic than 'Key 10' leads us to hypothesize that there might be some genes originating from the susceptible parent, 'Roblin', which in the presence of the antixenosis genes from 'Key 10' result in more deterrence. Another hypothesis might be the expressions of some genes in 'Key 10' which result in decreasing the effect of antixenosis genes.

If there was one gene conferring antixenosis, the lines would have segregated into half resistant and half susceptible. However, the frequency distribution of lines in the

field and laboratory indicates that there was more than one gene conferring antixenosis (Field 2006: susceptible (47 lines) : resistant (32 lines) : highly resistant (6 lines); Field 2007: susceptible (3 lines) : moderately resistant (38 lines) : resistant (48 lines) : highly resistant (1 line); laboratory: highly susceptible (2 lines) : susceptible (19 lines) : moderately resistant (17 lines) : resistant (49 lines) : highly resistant (3 lines) levels). As the tested lines were doubled-haploids, there were no heterozygotes among the lines, so the ratio of the lines in different categories regardless of the number of genes must be equal. We tried the method recommended by Choo and Reinbergs (1982*a*) for estimating the number of genes in a doubled-haploid population. However, the results were not meaningful and the number of genes estimated by their method was unreasonably high, e.g. 30 genes. However, the ratio of 25 (consistently resistant lines) : 67 (the rest of the lines) fits a 1 : 3 ratio ($\chi_1^2 = 0.2319$, $P = 0.6301$). This indicated that there are possibly two genes conferring antixenosis. A fast method which can be helpful in determining the number of genes and their location on DNA would be Bulk Segregant Analysis (Michelmore *et al.* 1991) which we have started; the results will likely be available in the near future. Studies on other pests have shown that there are usually two to four resistance genes against pests (Boozaya-Angoon *et al.* 1984; Amri *et al.* 1990; Pani and Sahu 2000).

The complementary or duplicate interactions of the antixenosis genes were estimable. The kurtosis coefficient was positive ($1.645 \pm 0.516 < g_2 < 2.99 \pm 0.502$), indicating that there were interactions among antixenosis genes. The positive value of the skewness coefficient ($1.042 \pm 0.261 < g_2 < 1.101 \pm 0.254$) indicated the nature of the interaction to be complementary, meaning that to have the highest antixenosis level, all

antixenosis genes must be present in the genome of the line. In the complementary gene interaction, the genes 'complement' the action of each other, e.g. physiologically or by production of chemical compounds, which result in higher levels of expression. Possibly, the antixenosis genes act together to produce deterrent volatiles (Chapter 2). A complementary gene interaction was found for genes conferring antibiosis against Hessian fly (Amri *et al.* 1990) and Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) (Diptera: Cecidomyiidae) (Pani and Sahu 2000).

In this study, the heritability of the antixenosis trait was estimated to be 67%. This level might be considered as good, but not high (above 85%), for breeding purposes. It may indicate that antixenosis against wheat midge can be developed by breeding resistant lines over several different crosses among wheat plants. It indicates that close to two-thirds of the antixenosis was affected by the genes which were transferred from one generation to the next generation. The effect of environment on genotypes was medium, based on the correlation coefficient of standardized egg density on lines in 2006 and 2007 field tests. This is the method developed by Falconer and Mackay (1996) for looking at the interaction of genotypes with two environments. If interaction was important, the correlations would be low; while correlations close to 1.0 would indicate no interaction. The medium or moderate effect of environment on antixenosis genes and final phenotypic expression may indicate that there is a need to screen wheat lines against wheat midge at more than one location. The effect of specific environmental factors on antixenosis genes can also be studied. It was found that environmental temperature affected the expression of genes conferring antibiosis against Hessian fly (Tyler and Hatchett 1993).

In summary, the screening of the doubled-haploid bread wheat lines showed that antixenosis to the wheat midge significantly decreased the egg density of the wheat midge in the field and laboratory, and can be considered as a method of controlling wheat midge. This might lead to a successful resistant-line breeding program, considering the 67% heritability. Based on our results, it is suggested that more than one gene with complementary interaction conferred antixenosis. These antixenosis genes were not linked to the antibiosis gene, *Sm1*. Also, spike length, spike compactness, and awnedness were not related to antixenosis. Other studies also showed that spike length are not related to the antixenosis level (Smith and Lamb 2001; Wise *et al.* 2001).

The lines which were consistently resistant in the laboratory and field can be useful in production of antixenotic lines. Application of antixenosis along with antibiosis may delay the evolution of virulent biotypes of wheat midge and may decrease the application of insecticides. This may benefit the growers financially, and decrease the side effects of insecticide applications on the environment. More research is needed to explore the interaction between resistant lines to wheat midge and natural enemies. Mapping the genes conferring antixenosis against wheat midge can be helpful in breeding resistant lines. The lines which were consistently antixenotic in this study can be tested with markers which are used for Hessian fly resistance genes. Some of the genes which confer antibiosis to Hessian fly may be the genes which confer antixenosis to wheat midge. It is hypothesized the antixenosis conferring genes produce the volatiles which are emitted by the antixenotic lines (Chapter 2).

Table 3.1. The symbols for antixenosis levels and corresponding definitions assigned for the doubled-haploid spring wheat lines used in screening for antixenosis against *Sitodiplosis mosellana* in the field and laboratory. 'Key 10' was the antixenotic bread wheat control line. 'Roblin' was the susceptible bread wheat control cultivar.

Symbol	Name	Definition
RR	Highly resistant	The mean egg density of the line was significantly lower than 'Key 10' based on Dunnett test.
R	Resistant	The mean egg density of the line was not significantly different from 'Key 10' based on Dunnett test.
M	Moderately resistant	The mean egg density of the line was between 'Roblin' and 'Key 10', and was significantly higher than 'Key 10' and significantly lower than 'Roblin' based on Dunnett test.
S	Susceptible	The mean egg density of the line was not significantly different from 'Roblin' based on Dunnett test.
SS	Highly susceptible	The mean egg density of the line was significantly higher than 'Roblin', based on Dunnett test.

Table 3.2. Correlations of number of eggs laid on the test lines, as proportion of eggs on 'Roblin', between laboratory and field screening tests against *Sitodiplosis mosellana*. 'Roblin' was the susceptible bread wheat control cultivar. Correlations are significant at $P < 0.05$.

	2007 field		laboratory	
	r_s	P	r_s	P
2006 field	0.406	0.0002	0.216	0.057
2007 field			0.436	<0.0001

Table 3.3. Log linear analysis of distribution of wheat midge eggs on different parts of a spikelet of spikes of wheat lines screened in the field and laboratory. Each test had a 3×2 contingency table classified by spikelet part and antixenosis level. Differences are significant at $P < 0.05$.

Environment	Effect	d.f.	Chi-Square	*P*
Field 2006				
	antixenosis level	1	4015.50	<0.0001
	Spikelet part	2	300.67	<0.0001
	antixenosis level×Spikelet part	2	171.00	<0.0001
Field 2007				
	antixenosis level	1	34567.20	<0.0001
	Spikelet part	2	5104.18	<0.0001
	antixenosis level×Spikelet part	2	1667.04	<0.0001
Laboratory				
	antixenosis level	1	13360.80	<0.0001
	Spikelet part	2	606.18	<0.0001
	antixenosis level×Spikelet part	2	77.01	<0.0001

Table 3.4. Log linear analysis of distribution of wheat midge eggs on three sections of a spike of wheat lines screened in the field and laboratory. Each test had a 3×2 contingency table classified by spike part and antixenosis level. Differences are significant at $P < 0.05$.

Environment	Effect	d.f.	Chi-Square	*P*
Field 2006				
	antixenosis level	1	4455.10	<0.0001
	Spike sections	2	542.87	<0.0001
	antixenosis level×Spike section	2	4.90	0.0863
Field 2007				
	antixenosis level	1	38970.00	<0.0001
	Spike part	2	5456.23	<0.0001
	antixenosis level×Spike section	2	5.76	0.0560
Laboratory				
	antixenosis level	1	30162.10	<0.0001
	Spike part	2	1981.54	<0.0001
	antixenosis level×Spike sectiont	2	78.76	<0.0001

Table 3.5. Analysis of variance of egg density of *Sitodiplosis mosellana* on spring wheat lines in the field, estimated for obtaining the broad sense heritability (h_b^2) for antixenosis trait.

Source	d.f.	Type I SS	Mean square	F value	*P*
Line	92	526.62	8.98	16.93	<0.0001
Year	1	2791.11	2791.11	5260.69	<0.0001
Line×Year	81	182.90	2.29	4.26	<0.0001

Table 3.6. Coefficients of skewness (g_1), kurtosis (g_2) and the respective standard errors estimated to explore the presence of interaction among antixenosis conferring genes against *Sitodiplosis mosellana* in the spring wheat lines screened in the field. See text for explanation.

Environment	g_1	g_2	SE g_1	SE g_2
2006 field	1.042	1.645	0.261	0.516
2007 field	1.101	2.990	0.254	0.502

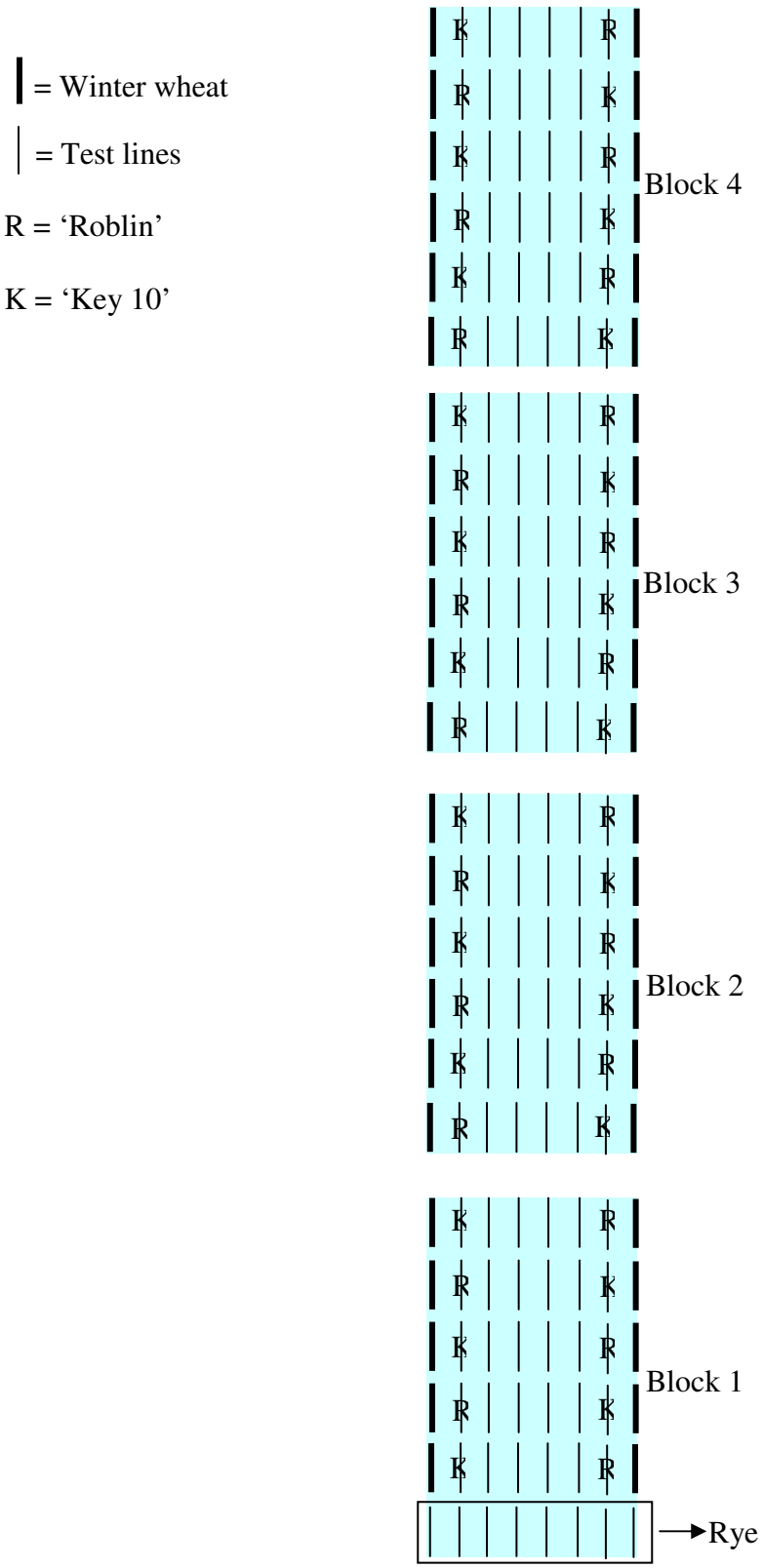


Figure 3.1. Schematic diagram of seeding pattern of wheat lines at Brandon in summer 2006 and 2007 used for screening lines against *Sitodiplosis mosellana.*

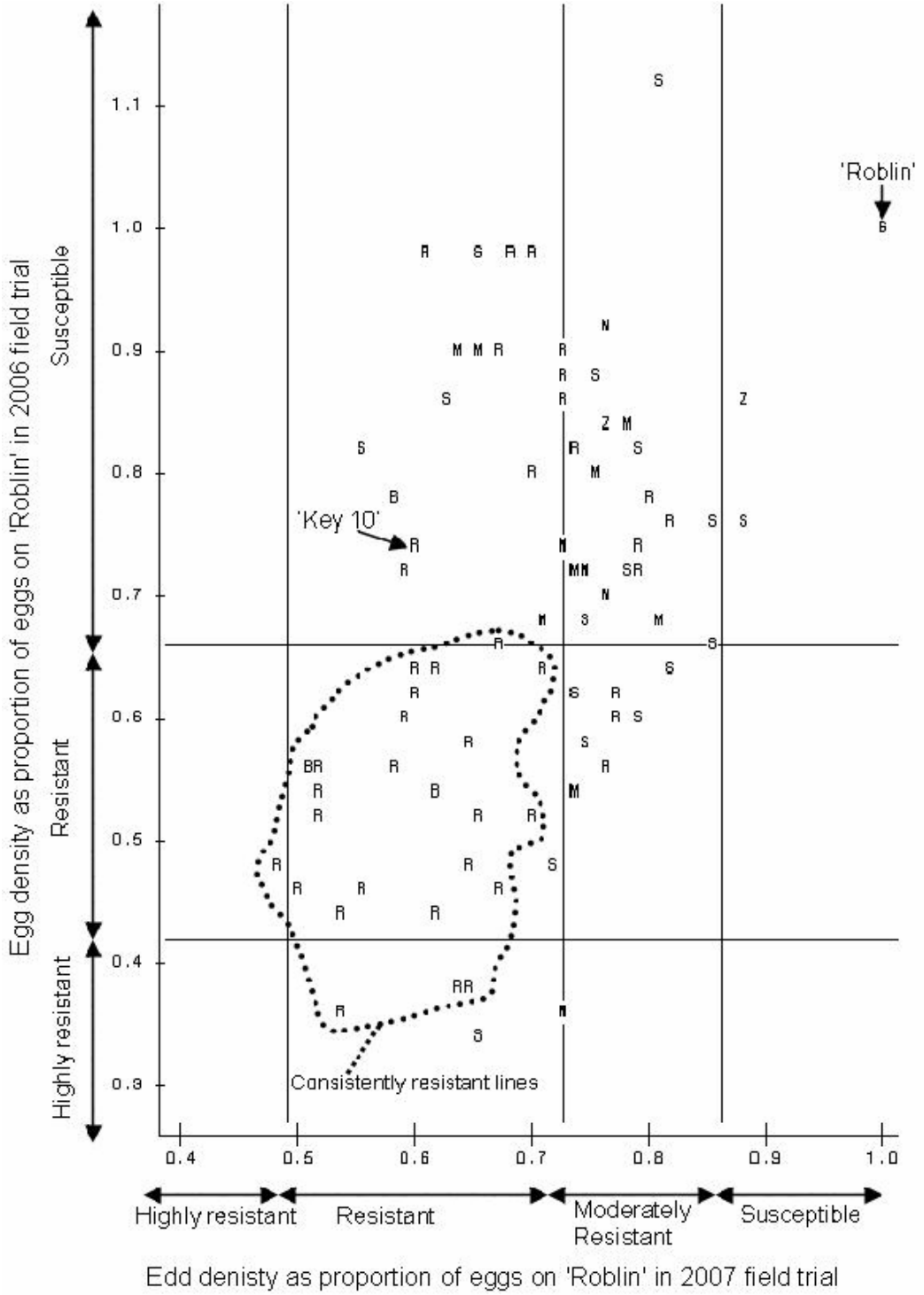
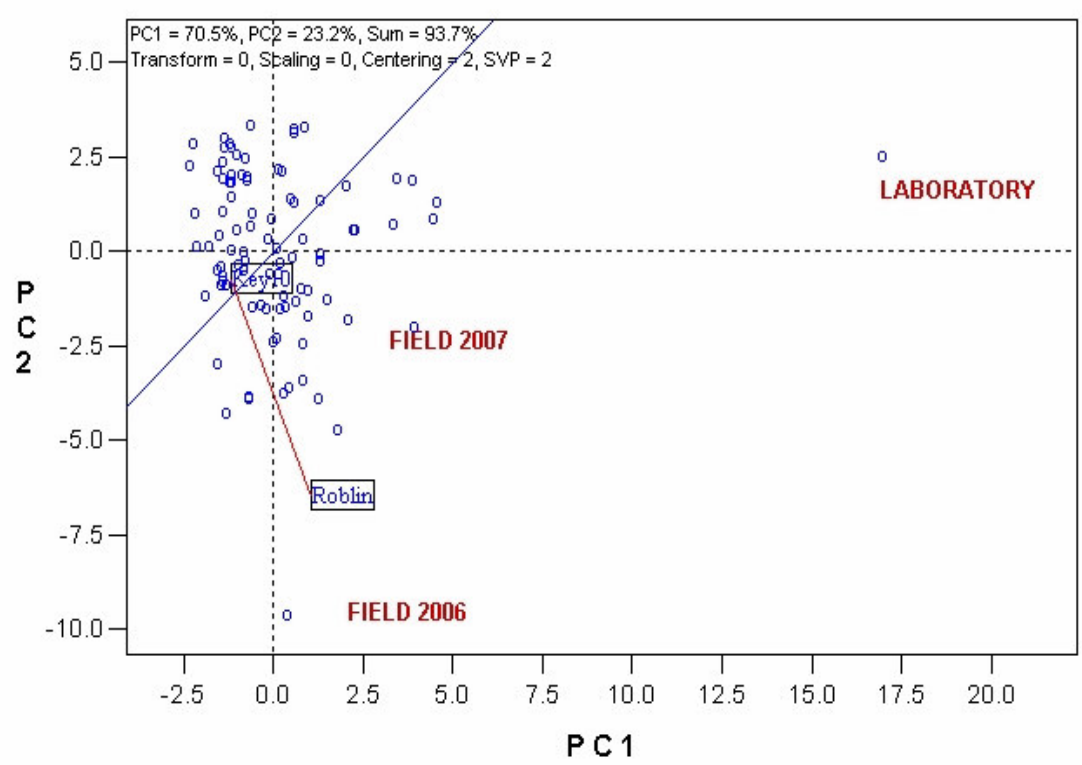


Figure 3.2. Relationship among egg densities of the lines in screening tests against *Sitodiplosis mosellana*. Each letter on the graph represents the resistance category of a wheat line based on the laboratory trial (Z = Highly susceptible, S = Susceptible, M = Moderately resistant, R = Resistant, B = Highly resistant). The y and x axes were divided into antixenosis levels based on the field trials.

a)



b)

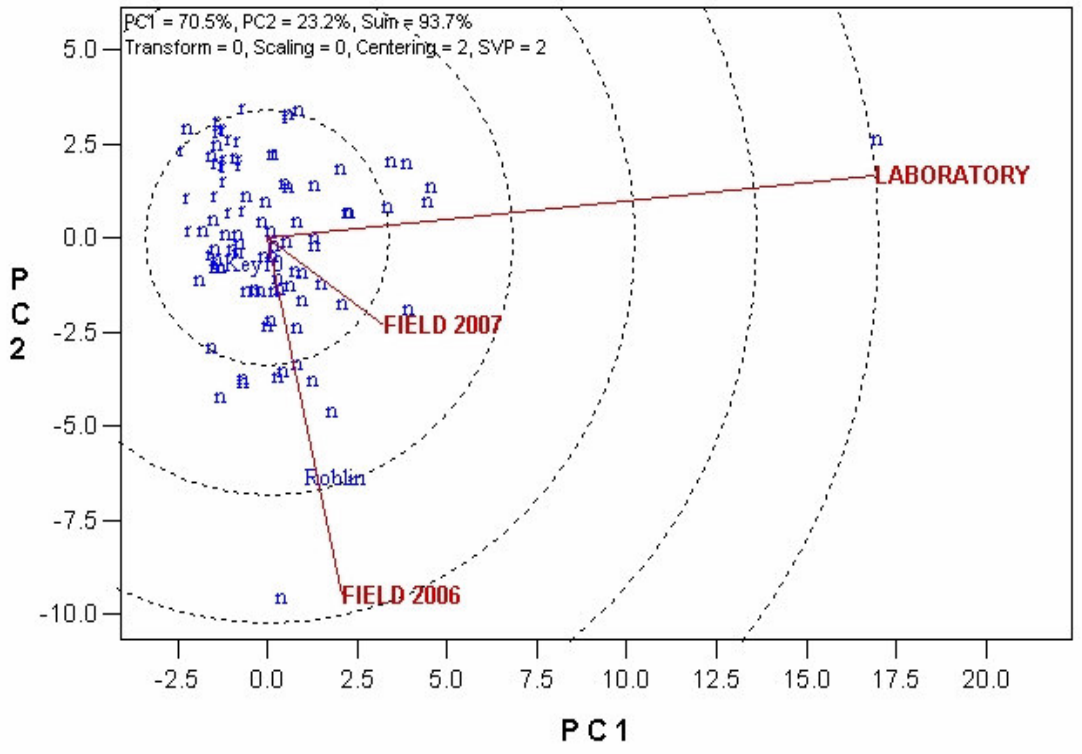


Figure 3.3. GGEbiplot analyses: a) comparing parent bread wheat lines of the doubled haploid population in the screening test for antixenosis against *Sitodiplosis mosellana* in the field and laboratory. b) comparing relationship among laboratory and field data (each symbol indicated a wheat line (r = Consistently resistant lines, n = the rest of the lines).

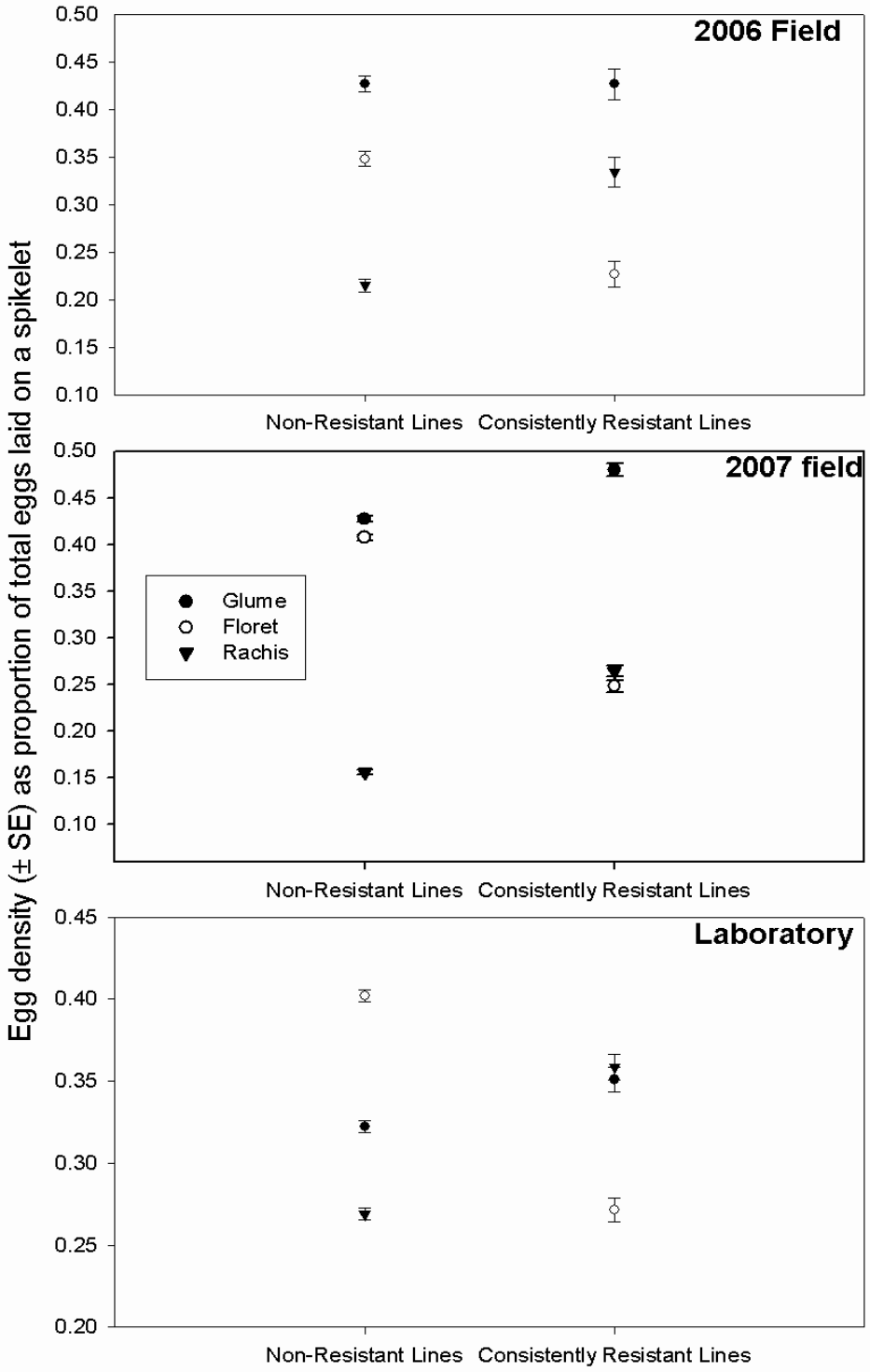


Figure 3.4. Proportion of eggs laid by *Sitodiplosis mosellana* on different parts of the spikelets of wheat lines in different antixenosis levels in the field and laboratory experiments.

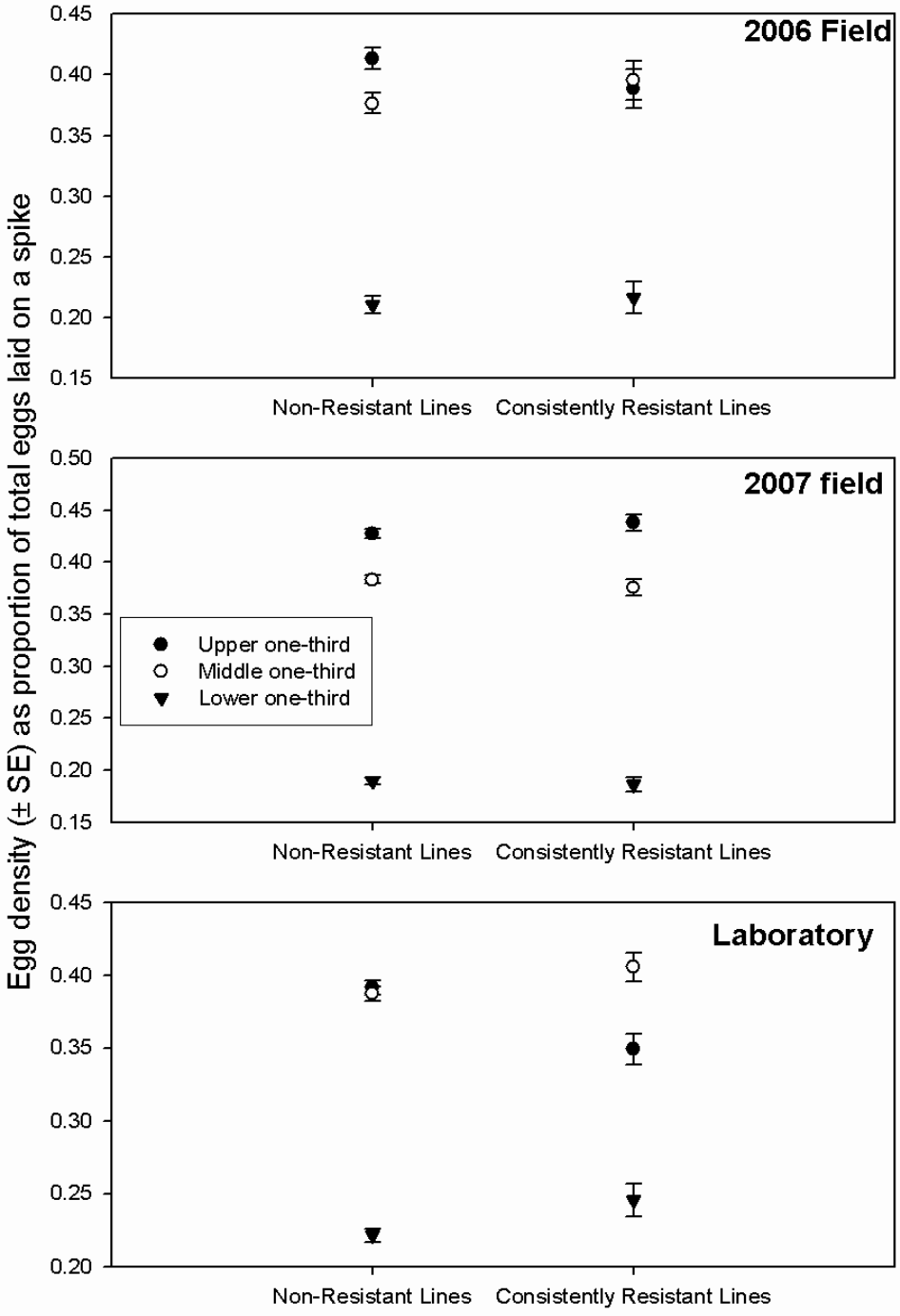


Figure 3.5. Proportion of eggs laid by *Sitodiplosis mosellana* on three sections of the spike of wheat lines in different antixenosis levels in the field and laboratory experiments.

Chapter 4

The relationship between morphological traits of the spring wheat spike and oviposition deterrence to wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), in the laboratory

Introduction

Exploring insect-plant interactions can be useful in production of resistant crops. If morphological traits of a plant cause resistance or are strongly correlated with resistance levels, those traits can be used by breeders as phenotypic markers in the selection processes to breed resistant crops (Sharma *et al.* 1990; Naik *et al.* 1996).

Wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), is a key pest of wheat in the northern Great Plains (Wright and Doane 1987; Lamb *et al.* 2000*a*). Females oviposit at dusk, from a short time before sunset until dark (Reeher 1945; Pivnick and Labbé 1993). Calm and humid weather is ideal for flight and oviposition (Reeher 1945; Barnes 1956; Basedow 1977*a*; Pivnick and Labbé 1993). Wheat midge oviposits on wheat spikes (Mukerji *et al.* 1988). Larvae feed on the surface of early-stage developing kernels (Ding and Lamb 1999) resulting in kernel shrivelling and reducing quality and quantity of yield (Miller and Halton 1961; Olfert *et al.* 1985). In addition to the widespread application of insecticide to control this pest (Elliott 1988*a*, 1988*b*), cultivars with antibiotic resistance to the wheat midge (Barker and McKenzie 1996; Ding *et al.* 2000; McKenzie *et al.* 2002) have been developed. Antixenosis against wheat

midge was found in some wheat lines. This resistance reduces oviposition rate by at least 50% and is suggested to be controlled by more than one gene (Lamb *et al.* 2000*a*, 2002*a*).

It is hypothesized that antixenosis against wheat midge might be related to the morphological traits of the wheat spike. Research on other cecidomyiids revealed the effect of tactile cues and physical traits on the oviposition of the female insects. For example, length of glume, lemma and lodicule of sorghum lines are associated with resistance to sorghum midge, *Contarinia sorghicola* Coq. (Diptera: Cecidomyiidae) (Sharma *et al.* 1990; Naik *et al.* 1996). Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), lays more eggs on the adaxial surface of wheat leaves compared to the abaxial surface; the adaxial surface have parallel grooves and ridges while the abaxial surface were smooth (Kanno and Harris 2000). Research on the effect of morphological traits of the spikes of 58 Chinese bread wheat lines (Ding and Guo 1993) on the oviposition of wheat midge showed that the angle between the spike axis and awn is related to antixenosis. However, physical traits and morphological characteristics of the spikes of 13 durum wheat lines, *Triticum durum* L. (Poaceae) (Lamb *et al.* 2001), did not reveal traits that affect wheat midge oviposition or could be used for indirect selection of breeding resistant lines. No research on the effect of morphological traits of bread wheat on oviposition rate by wheat midge has been undertaken. The objective of this study was to explore the relationship between the antixenosis levels of a set of related bread wheat lines, *Triticum aestivum* L. (Poaceae), and the morphological characteristics of the spikes.

Materials and Methods

In the laboratory, 92 doubled-haploid wheat lines and their parents, 'Roblin' and 'Key 10', were planted using the procedure described in Chapter 1. 'Roblin' is a susceptible bread wheat cultivar and 'Key 10' is an antixenotic bread wheat line. The sample size for each line was 10 pre-anthesis and 10 post-anthesis spikes. At heading stage, spikes which were 75% to 100% emerged from the flag leaves were sampled (Zadoks growth stage 57-59: Zadoks *et al.* 1974). The spikes were excised, bundled with tape, and labelled with the line number. For the lines that produced enough spikes at one time, 10 spikes were sampled at once. For the lines that produced fewer spikes at one time, sampling was done over several days. Some lines that did not germinate at all, or did not produce 10 spikes, were seeded again. In the laboratory, the traits described in Table 4.1 were measured for each spike and for the middle spikelet of each spike.

Spike features (Table 4.1) were measured under a stereomicroscope, which had an eye-piece micrometer with 100-subunits. The measuring unit of the eye-piece was calibrated using a micrometer slide (OB-M-2/200, Japan) marked with a 2 mm scale, divided into 200 subunits, each representing 0.01 mm.

The described traits were also measured after anthesis, except that post-anthesis measurements did not include the traits related to ligule and auricle. The post-anthesis samples were recognized by the dried-out anthers protruding out of the floret.

Statistical analysis

Data were analyzed using procedures of SAS® (SAS Institute Inc. 2002). Traits 2, 4, 9, 12 and 17 did not vary among lines, and so were not analyzed. For the rest of the

traits, data of pre-anthesis and post-anthesis stages were compared for each line using a parametric t-test (PROC TTEST), for normal data, or the equivalent non-parametric test, Wilcoxon-Mann-Whitney test (PROC NPAR1WAY), for non-normal data. To check the normality of variables prior to analysis, stem-leaf and normal probability plots were used (PROC PLOT). If less than 5% of the lines showed a difference between pre-anthesis and post-anthesis stages, data of pre-anthesis and post-anthesis stages were pooled. Otherwise, data from each stage were analyzed separately.

Data were square-root or log transformed, where necessary, to normalize distribution of data and distribution of residuals after analysis of variance, and remove heteroscedasticity of variances, so that assumptions of analysis of variance were met.

For the variables that were measured using a continuous unit, data were analyzed using a mixed model analysis of variance (PROC MIXED), with the corresponding variable as a dependent variable and the wheat lines or antixenosis level (Consistently resistant vs. non-resistant) as a fixed effect. The lines were grouped into two antixenosis levels based on the screening data presented in Chapter 3. Line (nested within antixenosis level) and spike were random effects of the models. Denominator degrees of freedom (d.f.) were estimated using the Satterthwaite method.

Ordinal traits (glume waxiness and shoulder shape), were analyzed by logistic regression modelling (PROC GENMOD: multinomial distribution, cumulative logit link function), with trait as the dependent effect and the wheat lines or antixenosis levels as independent effect. Comparison among antixenosis levels was done using the ESTIMATE statement.

Spearman correlation coefficients (r_s) among traits and also between each trait and the egg density for that line in the laboratory were calculated (PROC CORR). The egg densities were obtained from laboratory antixenosis screening results (Chapter 3). If there are environmental effects on the expression of the genes conferring morphological traits that affect oviposition, then it is appropriate to use oviposition and morphological data from the same environment, in this case laboratory data.

Differences among treatments were considered significant at $P < 0.05$ and the mean values are presented as the mean ± SE.

Results

The mean values of eight traits were different between the parents of the doubled-haploid progeny (Table 4.2). For some traits data were not significantly different between pre-anthesis and post-anthesis stages of each parent line, so for those traits, data of each line were pooled to provide a larger sample size. The following traits had significantly larger values in 'Key 10' compared to 'Roblin' (Table 4.2): ligule length, glume length, length of hair inside glume, palea length at post anthesis stage, length of hair inside lemma, length of hair at spikelet base, inter-spikelet distance, and length of hair at rachis edge at post-anthesis stage. 'Roblin' had longer hair length at the rachis edge at the pre-anthesis stage compared to 'Key 10' (Table 4.2). The shoulder shape did not differ between pre- and post-anthesis for 'Roblin' or 'Key 10'. The glume shoulder was a right angle (Figure 4.1; d: square) in 'Roblin', but in 'Key 10', the beak of the glume shoulder was slightly curved (Figure 4.1; c: rounded). In the pre-anthesis stage, 'Key 10' was waxier than 'Roblin' (Table 4.2). However, glume waxiness did not differ between

'Roblin' and 'Key 10' at the post-anthesis stage (Table 4.2); both of the lines were in category 3 of waxiness: wax covered most of the glume (Table 4.1).

In the doubled-haploid progeny, eight traits did not differ significantly between pre-anthesis and post-anthesis stages, so data for the stages were pooled. There were significant differences among lines for all of the measured traits (Table 4.3). However, there were no significant differences between the two antixenosis levels regarding the measured morphological traits (Table 4.3).

The egg density on each line was standardized as the proportion of egg density on the susceptible parent, 'Roblin', in the laboratory (Chapter 3). In the pre-anthesis stage, there were significant correlations between egg density in the laboratory and 10 of the traits out of 16 (Table 4.4). In the post-anthesis stage, there were significant correlations between egg density in the laboratory and 8 of the traits out of 14 (Table 4.4). In the pre-anthesis stage, the inter-spikelet distance (trait 20) had the highest correlation with egg density, followed by the length of hair inside lemma (trait 13) and glume length (trait 6). For the inter-spikelet distance, the r-squared value was $(-0.2867)^2 = 8\%$. In the post-anthesis stage, the inter-spikelet distance (trait 20) had the highest correlation with egg density ($r^2 = (-0.2726)^2 = 7\%$), followed by palea length (trait 14) and glume length (trait 6).

In both pre-anthesis and post-anthesis stages, there were significant correlations among some of the traits (Appendices 4 and 5). The highest correlations were between length of hair inside lemma (trait 13) and length of hair inside glume (trait 10) in pre-anthesis ($r_s = 0.6533$) and post anthesis ($r_s = 0.6211$) stages (Appendices 4 and 5). This was followed by correlations among glume length (trait 6), lemma length (trait 11) and

palea length (trait 14) in pre-anthesis ($0.4632 < r_s < 0.5768$) and post-anthesis stages ($0.4480 < r_s < 0.5288$) (Appendices 4 and 5).

There was a significant positive correlation between egg density and glume waxiness in the pre-anthesis stage, indicating that susceptible lines had waxier glumes than resistant lines (Table 4.4). There was no correlation with the glume waxiness at the post-anthesis stage (Table 4.4). Logistic regression showed no difference between the two antixenosis levels at pre-anthesis stage ($\chi_1^2 = 0.62$, $P = 0.4316$), but there was a difference at post-anthesis stage ($\chi_1^2 = 38.32$, $P < 0.0001$). Comparisons between the two antixenosis levels (Logistic regression: $\chi_1^2 = 27.44$, $P < 0.0001$) indicated that for the post-anthesis stage, lines in the non-consistently resistant category were 4.90 times more likely to have lower levels of glume waxiness than consistently resistant lines.

There was a significant positive correlation between egg density and shoulder shape in the pre-anthesis and post-anthesis stages, indicating that susceptible lines were more likely to have shoulder shapes that were right-angled or tipped (Figure 4.1; d and e) compared to resistant lines (Table 4.4). Logistic regression showed no difference between the two antixenosis levels at the pre-anthesis stage ($\chi_1^2 = 1.92$, $P = 0.1657$), but there was difference at the post-anthesis stage ($\chi_1^2 = 9.01$, $P = 0.0027$). Comparisons between the two antixenosis levels (Logistic regression: $\chi_1^2 = 9.09$, $P = 0.0026$) indicated that at the post-anthesis stage, consistently resistant lines were 1.83 times more likely to have a more rounded shoulder shape than the remaining lines.

Discussion

There were significant differences among the doubled-haploid spring wheat lines for the morphological traits of the spike. However, most differences were not significant among the antixenosis levels. The correlations among traits and egg density were significant for some traits, but the correlations were not sufficiently high to be predictive. The largest correlation coefficient was -0.2867 (inter-spikelet distance) which would have 8% predictability from the point of view of crop breeding, explaining one-twelfth of the variation in antixenosis level among lines. This does not mean that the correlated traits cannot be useful in studying the antixenosis to wheat midge. They could be the basis of new hypotheses which may clarify wheat midge oviposition behavior. Six traits which seemed to have implications for antixenosis are discussed below: glume waxiness, length of hairs inside glume and lemma, length of hair at rachis edge, inter-spikelet distance and shoulder shape.

The antixenotic parent, 'Key 10', was waxier in the pre-anthesis stage compared to the susceptible parent, 'Roblin'. However, in the pre-anthesis stage, the progeny lines susceptible to wheat midge were waxier compared to antixenotic lines. This might indicate that the wax genes (Post-Beittenmiller 1996) are not linked to the antixenosis conferring genes. If they were linked, it was expected that the resistant progeny lines would be waxier in the laboratory screening test, which was not the case. Moreover, the correlation between glume waxiness and egg density at pre-anthesis stage (0.1255), although significant, indicated that that just 2% of the variation in antixenosis level at pre-anthesis stage was explained by glume waxiness. However, the fact that consistently resistant lines were waxier than the rest of the lines at the post-anthesis stage, may

suggest that when antixenosis genes are together, the physiological processes that antixenosis genes control may be associated with the processes involved in production of wax. More research is needed to explore the effect of wax on oviposition of wheat midge. Another cecidomyiid, the Hessian fly, oviposits 25% to 100% more on leaves of non-waxy wheat genotypes compared to waxy ones (Cervantes *et al.* 2002).

The hairs inside glume and lemma were longer in 'Key 10' than in 'Roblin'. However, in the progeny of the cross, the length of hairs inside glume and lemma were not different between antixenosis levels, and correlation coefficients were not sufficiently high to be predictive for breeding purposes. In 'Roblin' the length of the hair at the rachis edge was longer compared to 'Key 10'. I hypothesize that there might be interactions among the length of hairs inside the glume, lemma and rachis edge of 'Roblin' and 'Key 10' and the volatiles of the spike (Chapter 2), and this interaction might affect the distribution of eggs on different parts of the spikelet of 'Roblin' and 'Key 10', but not the final antixenosis level. Wheat midge oviposited more eggs on the rachis compared to the glume and floret on 'Key 10', while on 'Roblin' wheat midge oviposited more on the glume and floret compared to the rachis (Chapter 2; Lamb *et al.* 2001).

Inter-spikelet distance between the middle spikelet and the spikelet just above it on the spike was longer in 'Key 10' and the screened resistant progeny lines in the laboratory compared to 'Roblin' and susceptible progeny. However, the correlation coefficient with egg density was not high (r_s = -0.2867) compared to promising traits of other plants recommended for breeding against other crop pests (Sharma *at al.* 1990; Naik *et al.* 1996). Moreover, the non-significant difference between consistently resistant and the rest of the lines regarding inter-spikelet distance did not make this trait promising

for breeding purposes. High correlation is needed to use indirect selection in breeding resistant plants. For example, selection for sorghum midge-resistant lines was suggested to be successful based on relationship between egg density of sorghum midge *C. sorghicola*, and glume length (r_s = 0.66), lemma length (r_s = 0.70) and lodicule length (r_s = 0.60) (Sharma *at al.* 1990; Naik *et al.* 1996).

The resistant parent line, 'Key 10', the resistant progeny lines screened in the laboratory, and consistently resistant lines had more rounded glume shoulder shapes, while the susceptible progeny lines screened in the laboratory, the susceptible parent, 'Roblin', and the non-consistently resistant lines had more square shoulder shapes. The correlations with antixenosis were significant, but the associations were low (Pre-anthesis r_s = 0.0842; Post-anthesis r_s = 0.1079), and it is hypothesized that the shoulder shape might not be biologically important in determining the deterrence level or be inherited along with antixenosis.

This study was done on bread wheat. Previous research on durum wheat, *Triticum durum,* showed that there were no significant correlations among morphological traits of the spikes of the durum wheat and antixenosis to wheat midge (Lamb *et al*. 2001). I hypothesized that there might be relationships among morphological traits of bread wheat and antixenosis to wheat midge, as bread wheat has one genome, D, more than durum wheat. It was based on the assumption that having more diverse genomes may increase the possibility of the presence of diverse genes, which might be linked to the antixenosis conferring genes. The study described here revealed that the studied morphological traits of bread wheat spikes are no more related to antixenosis than in durum wheat.

In summary, this study of morphological traits of the bread wheat lines indicated the possible effect of the hairs inside glume and lemma, and hairs at rachis edge on egg distribution pattern within spikelets of 'Roblin' and 'Key 10'. The correlations of some morphological traits with antixenosis were not sufficiently high to recommend their use as general phenotypic markers for breeding antixenotic wheat lines against wheat midge. However, more research is suggested to clarify the interaction between the morphological traits and wheat volatiles (Chapter 2), and their effect in combination on antixenosis levels. Studies of the fine scale properties of the plant surface and also exploring the possible receptors around the tip of the ovipositor of the wheat midge may clarify the effect of the morphological traits as tactile cues on wheat midge oviposition behavior.

Table 4.1. Morphological traits measured at pre-anthesis and post-anthesis stages for spikes of spring wheat lines (*Triticum aestivum* L.) grown in the laboratory.

Trait No.	Trait name (measured unit)	Method of measurement
1	Ligule length (mm)*	From base to top of ligule
2	Ligule hairiness	0, not hairy; 1, hairy
3	Auricle length (mm)*	From base to top of auricle
4	Auricle hairiness	0, not hairy; 1, hairy
5	Shape of glume shoulder	6 different shapes as shown in Figure 4.1
6	Glume length (mm)*	Length from base to shoulder top, not including beak
7	Glume width (mm)*	Width at glume mid-point from front edge to keel edge
8	Glume waxiness	0, not evident; 1, little; 2, moderate; 3, covering most of the glume
9	Hair inside glume	0, not hairy; 1, hairy
10	Length of hair inside glume (mm)**	Length from base to top of the longest hair at the upper half and inside glume
11	Lemma length (mm)*	Length from base of lemma to base of lemma
12	Hair inside lemma	0, not hairy; 1, hairy
13	Length of hair inside lemma (mm)**	Length from base to top of the longest hair on the upper half and inside the lemma
14	Palea length (mm)*	Length from base to top
15	Palea keel width (mm)*	Width of palea keel at middle section
16	Length of hair at palea edge (mm)**	Length from base to top of the longest hair at lower half of the palea edge
17	Hair at spikelet base	0, not hairy; 1, hairy
18	Length of hair at spikelet base (mm)**	Length from base to top of the longest hair at front base of the spikelet
19	Length of hair at rachis edge (mm)**	Length from base to top of the longest hair on the lower half of rachis edge
20	Inter-spikelet distance (mm)*	Length from base of the middle spikelet to the base of spikelet just above it
21	Inter-spikelet width (mm)*	Width of inter-spikelet part of rachis at the middle spikelet

* Measured to the nearest mm. ** Measured under stereomicroscope to the nearest 0.01 mm.

Table 4.2. Mean values of the morphological traits of the spikes of 'Roblin' (susceptible bread wheat cultivar) and 'Key 10' (antixenotic bread wheat line), parent lines of the doubled-haploid progeny used for screening for antixenosis against *Sitodiplosis mosellana*. Comparisons of the parents were based on Wilcoxon-Mann-Whitney test. Differences are significant at $P < 0.05$. See Table 4.1 for description of the traits.

	Trait values (mm) in parent lines				Differences between parent lines	
	'Roblin'		'Key 10'			
Trait	Stage	Mean±SE	Stage	Mean±SE	*Z*	*P*
Ligule length	Pre-anthesis	1.42±0.09	Pre-anthesis	2.00±0.05	-3.5979	0.0003
Auricle length	Pre-anthesis	2.72±0.36	Pre-anthesis	3.61±0.30	-1.7556	0.0792
Glume length	Pooled	7.15±0.17	Pooled	8.05±0.14	-3.6424	0.0003
Glume width	Pre-anthesis	3.90±0.10	Pooled	3.75±0.10	0.9202	0.3574
	Post-anthesis	3.40±0.16			-1.8174	0.0691
Glume waxiness	Pre-anthesis	8.1*	Pre-anthesis	12.9*	4.0533**	0.0441
	Post-anthesis	10.0*	Post-anthesis	11.0*	1.0000**	0.3173
Length of hair inside glume	Pooled	0.3±0.05	Pooled	0.5±0.04	-3.6424	0.0003
Lemma length	Pooled	9.5±0.2	Pooled	10.0±0.2	-1.5792	0.1143
Length of hair inside lemma	Pooled	0.33±0.05	Pre-anthesis	0.43±0.05	3.3392	0.0008
			Post-anthesis	0.56±0.04	3.9748	<.0001
Palea length	Pooled	9.50±0.11	Pre-anthesis	9.70±0.15	0.9990	0.3178
			Post-anthesis	10.50±0.22	3.3950	0.0007

* Mean scores are presented. ** Kruskal-Wallis test, chi-square result is presented. Continued.

Table 4.2 Continued.

	Trait values in parent lines				Differences between parent lines	
	'Roblin'		'Key 10'			
Trait	Stage	Mean±SE	Stage	Mean±SE	*Z*	*P*
Palea keel	Pre-anthesis	2.00±0.00	Pooled	1.80±0.07	2.0618	0.0392
	Post-anthesis	1.12±0.08			-3.9939	<0.0001
Length of hair at palea edge	Pooled	0.26±0.03	Pooled	0.23±0.01	0.8469	0.3970
Length of hair at spikelet base	Pooled	0.31±0.03	Pooled	0.40±0.02	-2.7811	0.0054
Length of hair at rachis edge	Pre-anthesis	0.70±0.03	Pre-anthesis	0.53±0.03	2.9263	0.0034
	Post-anthesis	0.56±0.03	Post-anthesis	0.67±0.03	-2.1392	0.0324
Inter-spikelet distance	Pooled	4.95±0.05	Pooled	6.05±0.13	-5.1755	<0.0001
Inter-spikelet width	Pooled	2.88±0.07	Pooled	2.73±0.08	1.7972	0.0723

Table 4.3. Comparisons among spring wheat lines, and between antixenosis levels against *Sitodiplosis mosellana*, based on morphological traits of wheat spikes grown in the laboratory. Differences are significant at $P < 0.05$. See Table 4.1 for description of the traits.

Trait	Stage**	Data transformation for ANOVA	Differences among lines		Differences between antixenosis levels		Trait mean (mm)±SE by antixenosis levels*	
			F	*P*	*F*	*P*	Consistently resistant	Non-resistant
1	Pre	-	$F_{81,691}$=18.65	<0.0001	$F_{1,81.9}$=0.83	0.3654	1.42±0.03	1.34±0.02
3	Pre	-	$F_{81,682}$=6.76	<0.0001	$F_{1,82.3}$=0.74	0.3907	2.93±0.07	2.77±0.04
6	Pooled	-	$F_{82,1426}$=11.54	<0.0001	$F_{1,83.6}$=0.15	0.6966	7.84±0.03	7.84±0.02
7	Pooled	-	$F_{81,1415}$=7.94	<0.0001	$F_{1,83.3}$=3.76	0.0558	3.62±0.03	3.73±0.02
10	Pre	Square-Root	$F_{82,727}$=21.95	<0.0001	$F_{1,83.2}$=0.84	0.3621	0.46±0.01	0.41±0.01
	Post	Square-Root	$F_{77,615}$=18.49	<0.0001	$F_{1,78.4}$=0.31	0.5810	0.40±0.01	0.39±0.01
11	Pooled	-	$F_{82,1428}$=12.23	<0.0001	$F_{1,83.3}$=0.89	0.3494	9.92±0.04	9.97±0.02
13	Pooled	Square-Root	$F_{82,1436}$=27.89	<0.0001	$F_{1,83.4}$=0.22	0.6397	0.55±0.01	0.54±0.01
14	Pooled	-	$F_{82,1431}$=12.86	<0.0001	$F_{1,83.6}$=0.00	0.9970	10.01±0.03	9.96±0.02
15	Pre	-	$F_{82,737}$=4.60	<0.0001	$F_{1,82.7}$=0.65	0.4242	1.81±0.02	1.84±0.01
	Post	-	$F_{77,616}$=5.62	<0.0001	$F_{1,75.7}$=1.30	0.2582	1.64±0.02	1.69±0.01
16	Pooled	Square-Root	$F_{82,1431}$=2.58	<0.0001	$F_{1,85.6}$=3.90	0.0515	0.18±0.01	0.21±0.01
18	Pre	-	$F_{82,725}$=18.08	<0.0001	$F_{1,83.2}$=0.03	0.8570	0.27±0.01	0.27±0.01
	Post	-	$F_{77,616}$=10.30	<0.0001	$F_{1,77.1}$=1.15	0.2874	0.23±0.01	0.26±0.01
19	Pooled	-	$F_{82,1421}$=6.87	<0.0001	$F_{1,81.9}$=0.37	0.5460	0.58±0.01	0.57±0.01
20	Pooled	Log	$F_{82,1432}$=16.99	<0.0001	$F_{1,83.7}$=1.90	0.1718	6.05±0.04	5.90±0.02
21	Pre	Log	$F_{82,726}$=10.05	<0.0001	$F_{1,83.3}$=0.35	0.5545	2.66±0.03	2.68±0.02
	Post	Log	$F_{77,609}$=8.39	<0.0001	$F_{1,76.9}$=0.02	0.8927	2.50±0.03	2.51±0.02

** Pre = Pre-anthesis stage, Post = Post-anthesis stage.

Table 4.4. Correlation between egg densities of *Sitodiplosis mosellana* on doubled-haploid wheat lines and morphological traits of wheat spikes, grown in the laboratory. Egg density was standardized as proportion of egg density on the susceptible parent line, 'Roblin'. Differences are significant at $P < 0.05$.

	Pre-anthesis stage		Post-anthesis stage	
Trait	r_s	P	r_s	P
Ligule length	-0.0192	0.5967	-	-
Auricle length	-0.1056	0.0036	-	-
Shoulder shape	0.0842	0.0189	0.1079	0.0050
Glume length	-0.1584	<0.0001	-0.2166	<0.0001
Glume width	0.0851	0.0178	-0.0201	0.6020
Glume waxiness	0.1255	0.0005	-0.0434	0.2596
Length of hair inside glume	0.1341	0.0002	0.1922	<0.0001
Lemma length	-0.0503	0.1611	-0.1645	<0.0001
Length of hair inside lemma	0.2208	<0.0001	0.1848	<0.0001
Palea length	-0.1173	0.0010	-0.2356	<0.0001
Palea keel width	0.0558	0.1199	0.0020	0.9595
Length of hair at palea edge	0.0313	0.3828	0.0256	0.5064
Length of hair at spikelet base	-0.0574	0.1101	0.0972	0.0119
Length of hair at rachis edge	0.0768	0.0325	0.0015	0.9696
Inter-spikelet distance	-0.2867	<0.0001	-0.2726	<0.0001
Inter-spikelet width	-0.0281	0.4351	0.0396	0.3048

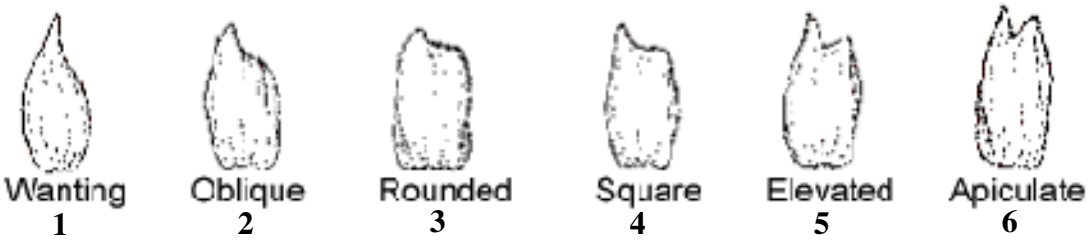


Figure 4.1. Shoulder shapes of glumes used to categorize wheat lines (Adapted from CFIA 2008).

Chapter 5

Oviposition behavior of wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), in the laboratory

Introduction

Insects search the environment for food, shelter, mates and oviposition sites. During the process of locating a suitable host plant, an insect is affected by exogenous and endogenous factors (Miller and Stickler 1984). Exploring these factors such as egg pressure, insect age, insect density, feeding (Hillyer and Thorsteinson 1969), environmental temperature (Rawlins 1980), humidity, wind, host plant density and host plant physiological status can reveal details of insect-plant interactions, in particular the cues used by an insect for host acceptance and oviposition. Research on these cues can be useful in breeding resistant lines against a crop pest.

Human agriculture often involves monocultures of plants which are attacked by insect pests. The interactions between insect pests and non-random plant distributions have been studied (Janzen 1970; Connell 1978) and can reveal how plants in a particular patch with a specific distribution are attacked by insects. In some cases, where the plants are abundant, they are attacked more (Singer 1971; Cullenward *et al.* 1979) and individual plants which are isolated escape discovery by the insect (Janzen 1970; Davis 1975). Research on the behavior of an insect, related to host distribution, can reveal how an insect allocates eggs and consumes the host plant, which might be useful in manipulating cropping systems and reducing pest damage.

Cereals such as wheat are major crops in agriculture, and research on cereal pests has been an important field for entomologists and breeders. Oviposition behavior and interactions with host plants were reported for cecidomyiid cereal pests such as sorghum midge, *Contarinia sorghicola* Coq. (Diptera: Cecidomyiidae) (Waquil *et al.* 1986); Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae) (Harris and Rose 1989), and wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) (Ganehiarachchi and Harris 2007). Sorghum midge behavior on resistant and susceptible lines revealed differences in probing and oviposition, which resulted in hypothesizing the possible factors for antixenosis (Waquil *et al.* 1986). In the case of Hessian fly, it was found that mating and environmental temperature influence the onset of oviposition (Harris and Rose 1991), and female Hessian flies use a combination of visual, chemical and tactile cues for host finding (Harris and Rose 1989, 1990). The effect of exogenous factors such as light (Hinks and Doane 1988), wind (Basedow 1977*a*), temperature (Pivnick and Labbé, 1993), and relative humidity (Pivnick 1993; Olfert *et al.* 1985) on wheat midge mating, oviposition and flight were studied. More study is needed to clarify the details of oviposition behavior and the physiological factors affecting host finding and egg allocation if we are to exploit antixenosis as a crop resistance strategy against wheat midge.

The wheat midge is a key pest of wheat in the northern Great Plains (Wright and Doane 1987; Lamb *et al.* 2000*a*). Females oviposit at dusk, a short time before sunset until dark (Reeher 1945; Pivnick and Labbé 1993) on wheat spikes (Mukerji *et al.* 1988). Larvae feed on the surface of early-stage developing kernels (Ding and Lamb 1999), resulting in kernel shrivelling, and reduction in quality and quantity of yield (Miller and

Halton 1961; Olfert *et al.* 1985). In addition to the widespread application of insecticides to control this pest (Elliott 1988*a*, 1988*b*), cultivars antibiotic to the wheat midge (Barker and McKenzie 1996; Ding *et al.* 2000) containing the antibiosis gene, *Sm1* (McKenzie *et al.* 2002), have been developed. Antixenotic lines were found (Lamb *et al.* 2000*a*, 2002*a*) which decrease wheat midge egg density on resistant wheat lines. Some aspects of oviposition behavior and the effect of resistant and susceptible host plants on the behavior of wheat midge were studied (Ganehiarachchi and Harris 2007). They found that wheat midges oviposit more eggs before midnight than after midnight, and oviposition rate does not differ between excised and non-excised wheat spikes. They also found that presence of male midges or higher densities of female midges in a confined arena increases wheat midge egg density on wheat spikes.

Antixenosis is usually more difficult to study than antibiosis because behavior of an insect is more difficult to quantify than survival or growth. Therefore, observations on wheat midge oviposition behavior can reveal unknown aspects of wheat midge host finding, host accepting and oviposition, which may help to understand how antixenosis works. The results can also help in designing appropriate procedures for screening wheat lines to detect antixenosis. The objectives of this study were to explore the differences in oviposition behavior of wheat midge on a susceptible spring wheat cultivar, 'Roblin', and an antixenotic line, 'Key 10' in the laboratory; to determine the relationship between the distribution pattern of 'Roblin' spikes in the laboratory and oviposition rate of wheat midge; to study the effect of wheat midge density in the laboratory on oviposition rate; and to determine the effect of the flag leaf on oviposition rate in the laboratory.

Materials and Methods

Effect of spatial distribution of spikes in the laboratory on oviposition rate of wheat midge

The experiment exploring the effect of spatial distribution of wheat spikes on wheat midge egg density consisted of five different tests (Table 5.1). 'Roblin' plants were seeded as described in Chapter 1. For Test 1, 30 spikes, at heading stage, were excised 45 cm from the tip of the spike. All leaves except the flag leaves were removed. Spikes were transferred to the test room, cut to a height of 40 cm and placed into piks as described in Chapter 2. Two arenas were constructed using 60 × 60 × 5-cm Styrofoam blocks, which were placed on two separate base frames, then covered with black plastic and a layer of soil to simulate the presence of soil in the field. The space inside a cage is referred to as an arena. At the centre of one arena, 15 of the spikes were positioned in two rows. The distance between two adjacent spikes was 5 cm. In the other arena, five spikes were placed touching each other at the points of a triangle. The distance between each two groups was 50 cm. Each group was 5 cm from the edge of the arena. The spikes and inside of the cages (60 × 60 × 60 cm) were misted with water and placed under fluorescent lamps. Into each arena, 15 female and 15 male wheat midges were released. After three nights, the cages were dismantled and spikes were dissected. The number of eggs laid on each spikelet was recorded. Arena set-up and dismantling date, number of wheat midges released, and treatment name were recorded. The cage was turned 90º between each of the six replications to control for any position effect.

The procedure for Test 2 (Table 5.1) was the same as Test 1, except that instead of having three 5-spike bundles, 15 spikes were positioned next to each other in a circular

cluster at the centre of the arena. As in Test 1, 15 female and 15 male wheat midges per arena were released.

The procedure for Test 3 (Table 5.1), which was a choice test, was similar to Test 1, except that instead of two arenas for each replication, there was one arena and each test was replicated 5 times. In each arena, the uniformly-spaced spikes were placed in two rows 7 cm from one edge of the arena, and on the other side 15 clumped spikes were positioned 7 cm from the other edge. Into each arena, 30 female and 30 male wheat midges were released.

The procedure for Test 4 (Table 5.1) was the same as Test 3, except that all flag leaves were removed. The procedure for Test 5 (Table 5.1) was the same as Test 3, except that 15 female and 15 male wheat midges per arena were released.

Behavioral studies

'Roblin' and 'Key 10' spring wheats were seeded as described in Chapter 1. In order to observe the oviposition behavior on 'Roblin' or 'Key 10', 5 spikes at the heading stage were excised at 50 cm from the terminal spikelet (not including awns). All leaves except the flag leaves were cut off. If the flag leaf was covering some of the spikelets, it was rolled back gently. The spikes were transferred into the test room, cut to the height of 45 cm, and inserted into the piks as described in Chapter 2. The spikes were numbered from 1 to 5. On a paper sheet, a schematic figure of a spike with 22 spikelets was drawn, and the spikelets of the figure were numbered sequentially from 1 to 22, starting from the bottom spikelet. The 'Roblin' spikes usually had 16 to 21 spikelets per spike. For each spike, the number of spikelets was recorded. The piks containing the spikes were placed

in a row, 3 cm from each other on a Styrofoam block, and the spikes were misted with water. At this time, the light of the test room was at the evening dusk setting with light level decreasing, conditions under which wheat midge normally oviposits in the field. The cage used was 55 cm tall and square shaped (25 × 25 cm), made of bamboo sticks with its walls covered with plastic cling film. About 20 female and male wheat midges (24-48 h old) were brought from the growth cabinet. Wheat midges older than 48 h were not used, as possible egg pressure could bias the results. Wheat midges were released into the arena, and flew upward toward the top of the arena, so it was easy to put the cage over the plants. A stop-watch was used to record the time and duration of wheat midge on-spike activities to the nearest second. As soon as one of the female wheat midges landed on a spike, recording was initiated, and the cage was removed to facilitate observation.

The movements of different parts of the body of the insect were recorded: moving the antennae, extending the ovipositor, resting (i.e. not moving or ovipositing) and walking on the spike. Preliminary observations followed by spike dissections indicated that when a wheat midge was ovipositing, she remained still and did not move while the ovipositor was inside the spikelet. Recognition of this behavior was helpful in identification of the spikelets in which she oviposited. While she was on the spike, her behavior was carefully observed to see when she laid the first egg. For each oviposition incident, when she inserted the ovipositor and remained still, the recorded time was named ‘start’, and when she retracted the ovipositor, the recorded time was named ‘finish’. For each oviposition incident, the start and finish times, and also the spikelet on which the eggs were laid were recorded. The observations ended when she flew away. The spike was dissected by removing and dissecting spikelets on which oviposition had

occurred, starting from the lowest-numbered spikelet. All parts of the spikelet were gently taken apart, and the total number of eggs laid on the spikelet was recorded. The procedure for 'Key 10' was the same as for 'Roblin', except that before dissection, the awns were trimmed to ease the dissection.

Statistical analysis

Data were analyzed using procedures of SAS® (SAS Institute Inc 2002). For the spatial distribution experiment, each spike was considered as a sample unit and the numbers of eggs on a spike were used in the analysis. The normality of data and also normality of residuals after analysis of variance were checked by stem-leaf and normal probability plots (PROC UNVARIATE). Homoscedasticity was tested by observing graphical distribution plots of variance by mean (PROC PLOT). When necessary, the data were square-root or log transformed to stabilize the variance. If these transformations could not remove heteroscedasticity, Taylor's Power Law was applied to find the appropriate power for transformation (Taylor 1961). A mixed model analysis of variance (PROC MIXED) was used to compare the egg numbers per spike for each treatment. Spike distribution was a fixed effect, and replication and spike were random effects. Denominator degrees of freedom (d.f.) were estimated using the Satterthwaite method.

For analyzing the effect of flag leaf (Tests 3 and 4), the numbers of eggs per female per replication were compared using a t-test (PROC TTEST). In addition, the numbers of eggs per spike for flag leaf effect were analyzed using a mixed model analysis of variance (PROC MIXED). Replication, spike and spike distribution (nested

within replication) were random effects. The assumptions of analysis of variance were tested as above.

For analyzing the effect of wheat midge density (Tests 3 and 5), the numbers of eggs per female per replication were compared using a t-test (PROC TTEST). In addition, the numbers of eggs per spike when midge density was decreased were analyzed using a mixed model analysis of variance (PROC MIXED). Replication, spike and spike distribution (nested within replication) were random effects. The assumptions of analysis of variance were tested as above. Denominator degrees of freedom (d.f.) were estimated using the Satterthwaite method.

In the oviposition behavior study, the total duration of on-spike activity was divided into 5-minute consecutive intervals, hereafter called time intervals. Before analysis, the durations of different activities were formatted as seconds.

The differences between total durations of on-spike activity on 'Roblin' and 'Key 10', and differences of total number of eggs laid on 'Roblin' and 'Key 10' were compared in each case by t-test (PROC TTEST).

To compare the differences between 'Roblin' and 'Key 10' in the duration from landing on a spike until laying the first egg, and also in the duration from laying the last egg until leaving the spike, a t-test was applied (PROC TTEST). For 'Roblin', the correlation between total number of eggs laid and total resting duration was tested (PROC CORR).

For 'Roblin' and 'Key 10', correlations between total on-spike duration and the following variables were tested: total number of eggs laid, duration from landing until laying first egg, and duration from laying the last egg until leaving the spike. For 'Roblin'

and ‘Key 10’, the linear regressions between total number of eggs laid (independent variable) and the following variables were tested: duration from landing until laying the first egg, and duration from laying the last egg until leaving the spike.

The data on observation of oviposition behavior were longitudinal data; that is, repeated measurements on a subject (wheat midge) over time; therefore the Repeated Measures Analysis (Wolfinger and Chang 1995) was done using PROC MIXED. Compound Symmetry (CS) covariance structure met the convergence criteria and also had high Akaike (AIK) and Schwarz (BIC) values, so it was chosen as the correct model. The dependent variables, which were analyzed in separate models, were duration of inter-oviposition intervals, duration for laying one egg, and total number of eggs laid per each time interval. For all three variables, the fixed effects were time interval, wheat line, and interaction between wheat line and time interval. The repeated statement in the model was assigned for the time interval. The subject was the wheat midge, or observation, as each observation was on one midge. By considering the time interval as a continuous variable, polynomial contrast was done by PROC MIXED models, using wheat line, wheat line × time interval, and linear, cubic and quadratic terms of time interval as fixed effects. Type I sums of squares was applied. Because the Type I *F*-test uses variance-covariance estimates from the full model, the parameters were re-estimated under a reduced model to gain more power in the model.

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

Results

The effect of spatial distribution of wheat spikes on oviposition rate of wheat midge in the laboratory

No-choice tests

In Test 1, assumptions of analysis of variance were met by log transformation of data. The average egg densities between 5-spike groups (45 ± 4) and uniformly spaced spikes (51 ± 4) were not different (MIXED ANOVA: $F_{1,173} = 0.67$, $P = 0.4125$) (Figure 5.1). In Test 2, assumptions of analysis of variance were met by square-root transformation of data. The average egg densities between clumped (40 ± 3) and uniformly spaced spikes (34 ± 4) were not different (MIXED ANOVA: $F_{1,159} = 3.24$, $P = 0.0737$) (Figure 5.1).

Choice tests

In Test 3, assumptions of analysis of variance were met using Taylor's Power Law for transformation of data (power for transformation = 0.175). The average egg densities between clumped (42 ± 4) and uniformly spaced spikes (77 ± 7) were different (MIXED ANOVA: $F_{1,130} = 16.12$, $P < 0.0001$) (Figure 5.2). In Test 4, assumptions of analysis of variance were met by square-root transformation of data. The average egg densities between clumped (45 ± 5) and uniformly spaced spikes (87 ± 7) were different (MIXED ANOVA: $F_{1,134} = 33.34$, $P < 0.0001$) (Figure 5.2). In Test 5, assumptions of analysis of variance were met by square-root transformation of data. The average egg densities between clumped (22 ± 3) and uniformly spaced spikes (34 ± 3) were different (MIXED ANOVA: $F_{1,130} = 10.90$, $P = 0.0012$) (Figure 5.2).

The effect of flag leaf on oviposition rate of wheat midge in the laboratory

The distributions of number of eggs per female per arena (replication) in Tests 3 and 4 were normal, justifying the use of a parametric t-test. The number of eggs per female in Test 3 (60 ± 10) and Test 4 (66 ± 4) were not different (d.f. = 8, t = -0.62, P = 0.5543).

Assumptions of analysis of variance of the number of eggs per spike were met by square-root transformation of data. The mean number of eggs per spike in Test 3 (60 ± 4) and Test 4 (66 ± 5) were not different (MIXED ANOVA: $F_{1,275} = 1.41$, $P = 0.2356$).

The effect of wheat midge density on oviposition rate of the female midges

The distributions of number of eggs per female per arena (replication) in Test 3 and Test 5 were normal, justifying the use of a parametric t-test. The number of eggs per female between Test 3 (60 ± 10) and Test 5 (57 ± 10) did not differ (d.f. = 8, t = -0.21, P =0.8379). Assumptions of analysis of variance of the number of eggs per spike were met by log transformation of data. The average number of eggs per spike between 30 midges per arena (60 ± 4) and 15 midges per arena (28 ± 3) was different (MIXED ANOVA: $F_{1,275} = 69.94$, $P < 0.0001$).

Behavioral observation

Description of the oviposition behavior of wheat midge in the laboratory

The following descriptions are the same for ‘Roblin’ and ‘Key 10’, except where a difference is noted. Wheat midges approached wheat spikes in several different ways (Figure 5.3). A wheat midge sometimes approached directly toward the spike and landed

on it. Sometimes she showed a zigzag flying pattern around the stem and finally landed on the spike or on the flag leaf. Sometimes she directly approached the stem and landed on it. In some cases, after landing on the plant, she moved her head near water droplets on the surface of the plant and appeared to drink. In this situation she did not antennate and the ovipositor was not extended. After drinking from water droplets, she either exhibited oviposition behavior, or rested on the plant and eventually flew away.

The movement of the wheat midge up and down the spike, and the spikelets on which she oviposited are shown in Figures 5.4 and 5.5 for 'Roblin' and 'Key 10', respectively. On 'Roblin', in 20 cases out of 27, she started oviposition from the lower spikelets of the spike and continued upward. On 'Key 10', this pattern occurred in 12 cases out of 22. When the female oviposited after landing on a spike, the behavior was as shown schematically in Figure 5.6. After landing on the spike, she stayed motionless (Figure 5.6.a) and did not visibly move parts of her body for a few seconds. Gradually, she extended her ovipositor (Figure 5.6.b) and lowered the tip of the ovipositor toward the surface of the spike (Figure 5.6.c), tapping on the surface and moving her ovipositor from side to side (Figure 5.6.d). While she was moving her ovipositor in this way, she started antennating at a rate of about 2 times per second. Counting the number of antennations was difficult, so no comparisons of antennation rate between 'Roblin' and 'Key 10' could be obtained. While antennating and moving her ovipositor, her body was kept close to the surface of the spike. While she was moving on the spike, she tapped on the surface with her forelegs. She laid eggs on the rachis, inside the glumes and inside the florets. Before laying eggs inside glumes, she inserted the tip of her ovipositor between the upper edge of glume and lemma and tapped the tip of her ovipositor to the inside of

the glume and surface of the lemma. She inserted her ovipositor further into that gap (Figure 5.6.e), while her legs were on the surface, moving her body and wings from side to side. She remained still and did not antennate while her ovipositor was inside the gap. After oviposition, she moved her body from side to side and retracted her ovipositor from the gap. Preliminary studies indicated that when she remained motionless while her ovipositor was inside the spikelet, she was laying eggs. While laying eggs on the rachis, her legs were on the lateral parts of the spikelets, she bent her ovipositor about 90º toward the rachis, moved her body from side to side, then remained still and did not antennate. After oviposition, she moved her body from side to side and retracted her ovipositor. For oviposition inside florets, she stayed on the outer surface of the spikelet, bent her ovipositor about 90º toward the bottom of the spikelet, where the edges of the two glumes join, and inserted her ovipositor into that gap, moved her body from side to side, then remained still and did not antennate. After oviposition, she moved her body from side to side and retracted her ovipositor.

When a wheat midge was probing and ovipositing on ‘Roblin’, she sometimes rested on the spike after activity; all landings on ‘Roblin’ resulted in oviposition. However, when she started probing and ovipositing on ‘Key 10’, she did not rest afterwards. After landing on ‘Key 10’ spikes, in 43 cases, she remained still and did not move; she flew away after a few minutes, ranging from 0.3 to 7.5 minutes in 40 cases and 9.3, 10.75 and 14 minutes in the extreme cases, without ovipositing.

When a female was walking on the spike and reached the terminal spikelet, she tapped her front legs a few times in the air, and then she turned and walked to the lower parts of the spike. Most of the oviposition incidents were observed while she was walking

from the bottom part of the spike upward; however, there were a few cases when she laid eggs while she was walking from the top of the spike downward. In most of the cases, when she was ovipositing, her head was upward and toward the tip of the spike, and in a few rare cases, her head was toward the bottom of the spike.

On the awned line, 'Key 10', sometimes a female positioned her legs on the awns, and stretched the ovipositor toward the spikelet, but the tip of the ovipositor did not reach the gaps.

After the last oviposition, a female remained still on the spike, retracted her ovipositor and did not antennate. After a while she flew away and the observation was ended. In some cases, she flew far from the spike and did not return; however, in some cases she showed a zigzag flight at a distance of about 30-40 cm from the spike on which she oviposited and then came back to the same region. If she came back and landed on another spike, observation was not continued; so just one spike per observation was considered.

Quantitative differences of several aspects of oviposition behavior on 'Roblin' and 'Key 10' bread wheats

The distributions of total on-spike duration and total number of eggs laid were normal for 'Roblin' and 'Key 10', justifying the use of a parametric t-test. Wheat midge females stayed 1.75 times longer on 'Roblin' (2068 ± 191 seconds, ≈ 35 minutes) than on 'Key 10' (1178 ± 132 seconds, ≈ 20 minutes) (d.f. = 44.3, t = -3.83, P = 0.0004). Wheat midge females laid 2.5 times more eggs on 'Roblin' (25 ± 2) than on 'Key 10' (10 ± 1) (d.f = 40.9, t = -5.73, $P < 0.0001$).

The distributions of duration from landing on spike until laying the first egg were normal for 'Roblin' and 'Key 10', justifying the use of a parametric t-test. Mean duration from landing on a spike until laying the first egg on 'Roblin' (89 ± 14, ≈ 1.5 minutes) and 'Key 10' (243 ± 37, ≈ 4 minutes) differed (d.f. = 40.9, t = -5.73, $P < 0.0001$). The distribution of duration from laying the last egg until leaving the spike was not normal for 'Roblin', but it was normal for 'Key 10'. Therefore, Wilcoxon-Mann-Whitney test was applied, which indicated that duration from laying the last egg until leaving the spike on 'Roblin' (Non-parametric mean score: 20.24; Mean ± SE: 113 ± 29, ≈ 1.8 minutes) and 'Key 10' (Non-parametric mean score: 30.84; Mean ± SE: 160 ± 23, ≈ 2.5 minutes) differed (Z = 2.57, P = 0.0101).

On 'Key 10', wheat midge did not show resting behavior after oviposition, so there were no data to analyze. However, on 'Roblin', females sometimes showed resting behavior while they were on the spike and between oviposition incidents. The distribution of total resting duration was not normal; however, the distribution of total number of eggs laid was normal. There was no correlation between total number of eggs laid on 'Roblin' and total duration of resting on spike (r_s = -0.150, P = 0.5502).

On 'Key 10', the distributions of total number of eggs laid and total on-spike duration were normal. There was a positive correlation (r_p = 0.76, $P < 0.0001$) between total number of eggs laid on 'Key 10' and total on-spike duration. Based on regression analysis, duration from landing until laying the first egg was not dependent on the total number of eggs laid ($F_{1, 20}$ = 0.01, P = 0.9164, adjusted r^2 = -0.0494, b = 0.66). Based on regression analysis, the duration from laying the last egg until leaving the spike was not dependent on the total number of eggs laid ($F_{1, 20}$ = 0.01, P = 0.9157, adjusted r^2 =

-0.0494, b = -0.41). There was a correlation between total on-spike duration and duration from landing on spike until laying the first egg (r_p = 0.49, P < 0.0193). There was no correlation between total on-spike duration and duration from laying the last egg until leaving the spike (r_p = 0.13, P = 0.5479). The distributions of total on-spike duration, duration from landing on spike until laying the first egg, and duration from laying the last egg until leaving the spike were normal.

On 'Roblin', the distributions of total number of eggs laid and total on-spike duration were normal. There was a correlation (r_p = 0.65, P = 0.0002) between total number of eggs laid on 'Roblin' and total on-spike duration. Based on regression analysis, duration from landing on the spike until laying the first egg was not dependent on the total number of eggs laid ($F_{1, 25}$ = 1.03, P = 0.3209, adjusted r^2 = 0.0010, b = 1.25). Based on regression analysis, the duration from laying the last egg until leaving the spike was not dependent on the total number of eggs laid ($F_{1, 25}$ = 0.97, P = 0.3334, adjusted r^2 = -0.0010, b = -2.46). There was no correlation between total on-spike duration and duration from landing on a spike until laying the first egg (r_p = 0.02, P = 0.8874). There was no correlation between total on-spike duration and duration from laying the last egg until leaving the spike (r_s = 0.07, P = 0.7018). The distributions of total on-spike duration and duration from landing on spike until laying the first egg were normal. However, the distribution of the duration from laying the last egg until leaving the spike was not normal.

Considering the time interval as a continuous variable, polynomial analysis indicated that the linear-term contrast, which had significant P values, was appropriate compared to quadratic and cubic terms, for analyzing the three variables: duration of

inter-oviposition intervals, total number of eggs laid during 5-minute time intervals, and duration for laying one egg. The reduced model with just linear terms (Table 5.2) indicated that there were significant differences among time intervals, and between wheat lines regarding the total number of eggs laid during consecutive time intervals. However, the results for the other two variables: duration of inter-oviposition intervals, and duration for laying one egg, did not show an effect of the wheat line (Table 5.2). As time passed, duration of inter-oviposition intervals increased in both 'Roblin' and 'Key 10' (Figure 5.7). However, as time passed, duration for laying one egg did not change either on 'Roblin' or on 'Key 10' (Figure 5.7).

Discussion

Experiments on the effect of host patchiness and conspecific density on oviposition rate of wheat midge revealed new aspects of the interactions between wheat midge and the host plant. The continuous observations on wheat midge oviposition behavior showed that the wheat midge reacts differently on the susceptible and antixenotic wheats. Different aspects of oviposition behavior such as on-spike duration, total eggs laid, and inter-oviposition intervals differed between the susceptible and antixenotic wheats. The possible implications of these differences will be discussed.

Change in conspecific density from one female midge per two spikes to one female midge per one spike did not affect the oviposition rate of wheat midge in the laboratory. It was hypothesized that there might be some interactions among the midges while searching for a host plant in a confined space or probing a spike and ovipositing on it; so, by changing the number of midges per spike, the number of eggs per female might

change. We conclude that by increasing the midge density in a confined space, physiological or behavioral processes of oviposition were probably not affected although the probability that females encounter each other increased. By increasing the midge density, for example to three or four female midges per spike in an arena, there may be an effect of midge density on the oviposition rate. Our study was not intended to test whether the presence of a female midge attracts other midges to the same spike, as was observed by others in the field (Reeher 1945) or was suggested based on the laboratory observations (Ganehiarachchi and Harris 2007). The latter study showed that the presence of four female midges in a confined space compared to one female midge increased the presence rate of female midges on wheat spikes. This difference with our study might be due to the size of the cage used; in our study the cage was a 60-cm cube containing 30 spikes, but in the study done by Ganehiarachchi and Harris (2007) the cage was a glass cylinder with 17 cm height and 4 cm diameter, containing one wheat spike. The small glass cylinder possibly increased the contact rate of wheat midges more compared to our study.

The removal of the flag leaf did not affect the egg density on spikes of 'Roblin' spring wheat in the laboratory. It was hypothesized that the flag leaf of wheat might produce arrestant chemicals. Some plant tissues have arrestant chemicals (Dethier *et al.* 1960) which cause insects to aggregate on those tissues and slow down their movement. The possible arrestant compounds of the flag leaf might arrest the wheat midges resulting in a lower oviposition rate on the spikes, because the midge would stay more on the flag leaf and spend less time on spikes. Also, removal of flag leaf, which was hypothesized to be a resting place for midges, might cause midges to land on the spikes more, resulting in

an increase in contact time of wheat midge with spike volatiles. This might increase the oviposition rate. Our study did not support these hypotheses. We also tried to cover the flag leaves with cling film, so the wheat midge would not have contact with the flag leaf, but the midges stuck to the film and the experiments were not successful. More research is needed to explore the effect of wounding on the wheat plants to determine if excising the flag leaf results in production of specific chemicals in wheat. It was hypothesized that these chemicals might affect wheat midge oviposition behavior. Our study does not support this hypothesis; also previous research indicated that oviposition rate of wheat midge does not differ between spikes which are not excised from the plant and spikes which are excised from the plant (Smith and Lamb 2001; Ganehiarachchi and Harris 2007).

The effect of spatial distribution of spikes on wheat midge oviposition rate revealed new aspects of egg allocation by the female midges. In no-choice tests of the spatial distribution experiment, egg density per spike did not differ significantly between 'uniformly spaced' and 'three 5-spike groups', and between 'uniformly spaced' and 'clumped' treatments. However, in choice tests the egg density on the clumped spikes was significantly less than uniformly spaced spikes. In our no-choice tests, considering a female was confined for three nights, it is suggested that her oviposition behavior was as follows: she oviposited on a spike, flew away from that spike and oviposited on a new spike. So, it is suggested that a wheat midge flies away from the spike on which she has oviposited before beginning to oviposit again. Therefore, in a clumped patch she flies over some of the spikes in the clump before accepting an individual spike, taking her away from the clump. This might explain the higher egg density on uniformly spaced

spikes compared to clumped spikes in the choice test. However, in a no-choice test, the spikes were the only available resource, and they could exploit all of them during the three nights of the experiment.

It is hypothesized that in the field, wheat midge may show the same behavior as in the laboratory. A female may fly away from the spike on which she has already oviposited, before searching for a new spike. There might be a trade-off between the energy used for flying greater distances and increased fitness resulting from laying eggs on dispersed spikes. This might evolve due to the interaction with parasitoids. Probably, when parasitoids of wheat midge encounter wheat midge eggs, they search around that specific point more, compared to more distant spikes. Predators and parasitoids usually aggregate in patches of high prey density (Read *et al.* 1970; Evans 1976; Hassell 1978; Waage 1979).

A second evolutionary advantage for distributing eggs among dispersed spikes is related to the sex of the progeny of the wheat midge, which are either all-male or all-female for an individual adult female (Stuart and Hatchett 1991; Smith *et al.* 2004*b*), and the sedentary condition of the larvae. If a female lays all eggs in one place, there would be less chance for the progeny to find mates, as adults mate before dispersal (Pivnick and Labbé 1992). By distributing eggs on many spikes, the chance of finding a mate in a shorter time may increase. The close-to-even sex ratios (54-57% female) throughout experimental field plots (Smith *et al.* 2004*b*), in spite of single-sex families, might be corroboration for this suggestion.

A third evolutionary advantage for dispersed oviposition could be that by ovipositing on dispersed spikes wheat midges decrease the risk that all hosts in a

particular patch are unsuitable for their larvae. Wheat plants which grow in a particular place may be exposed to the same environmental conditions such as soil nutrients, water and light. The conditions might be poor for that patch, so if a wheat midge laid all eggs on that patch, it would increase the risk of mortality.

Another objective of this research was the continuous observation of oviposition by wheat midge on susceptible and antixenotic wheat lines under laboratory conditions in which decreasing light was used to simulate as in the field at dusk. As it was also reported by Ganehiarachchi and Harris (2007), in spite of the small size of a wheat midge and her nocturnal oviposition behavior, details of oviposition behavior were observable. It was found that observations could be done more easily by removing the cage after the wheat midge that landed on a spike and started oviposition. Once a female located a host plant, a consistent pattern of behavior occurs. Two behaviors were consistently observed while wheat midges were on the spikes: antennation and ovipositor tapping on the surface. The antennation behavior of wheat midge after landing on the spike is consistent with the conclusion that plant volatiles are important in host selection. The antennae of cecidomyiids are covered with looped hair-like structures called circumfila (Slifer and Sekon 1971), which are thought to have an olfactory function. The ovipositor-dragging behavior on the surface of the wheat spikes may indicate that chemical or tactile receptors around the tip of the ovipositor are used to detect cues for host suitability. In a cecidomyiid, *Dasineura brassicae* Winn., tactile sensors were found around the oviduct (Hallberg and Åhman 1987). Antennation and movement of the ovipositor on the surface of the host plant were observed for Hessian fly, *M. destructor* (Harris and Rose 1989, 1990), and in another behavioral observation study of wheat midge done in natural light

conditions (Ganehiarachchi and Harris 2007). The general ovipositional activities of the wheat midge were similar to what was observed for sorghum midge (Waquil *et al.* 1986) and also were the same as the observations done on wheat midge under natural light conditions (Ganehiarachchi and Harris 2007). Probing a spikelet with the ovipositor and remaining still during oviposition were observed for wheat midge and sorghum midge. However, in the case of wheat midge the downward movement of the eggs was not observed, while it was observed for sorghum midge (Waquil *et al.* 1986).

Our research explored quantitatively several aspects of oviposition behavior of wheat midge. First, the shorter duration from landing on the spike until laying the first egg on 'Roblin' compared to 'Key 10' may indicate that there are some stimulant volatiles on 'Roblin' and some deterrent volatiles on 'Key 10'. For sorghum midge, it was observed that females probed the susceptible lines for a shorter period before first oviposition, compared to resistant sorghum lines (Waquil *et al.* 1986). Second, the lower number of eggs on 'Key 10' and shorter on-spike duration on 'Key 10' compared to 'Roblin' might be due to deterrent volatiles of 'Key 10' (Chapter 2). Lower rates of probing and shorter on-spike duration on pre-anthesis spikes of 'Key 10', compared to 'Roblin', observed in a research done under natural light conditions (Ganehiarachchi and Harris 2007), might be due to deterrent volatiles. Third, the lack of resting behavior after oviposition on 'Key 10' may corroborate the effect of deterrent volatiles (Chapter 2). Fourth, on 'Roblin', as time went by the number of eggs laid decreased while on 'Key 10' there was no such relationship. This may indicate that 'Roblin' produced stimulatory cues which made wheat midge lay most of her eggs at the earlier part of on-spike duration. Fifth, on 'Roblin' and 'Key 10', time for laying one egg did not differ during

consecutive time durations, indicating that when a female oviposited, the rest of the process did not depend on the host; it probably was related to physiological processes related to the movement of eggs toward the tip of the ovipositor.

In conclusion, wheat midge oviposition rate was not affected by excising the bread wheat flag leaf or by increasing the midge density in a confined arena from one female midge per two spikes to one female midge per spike. On non-antixenotic wheat, the wheat midge stays longer, starts ovipositing sooner, lays more eggs, and leaves the spike sooner after the last oviposition. On antixenotic wheat, wheat midge stays for a shorter period, starts oviposition after a longer delay, lays fewer eggs, and leaves the spike after a longer delay from 23the last oviposition. The differences of egg density and different aspects of oviposition behavior, after landing on a spike, between the susceptible and resistant wheat lines are suggested to be due to deterrent volatiles of the antixenotic line or lack of stimulants on the antixenotic line. The details of oviposition behavior, reported in our study, which was done in the laboratory with field-simulated lighting conditions, were similar to what Ganehiarachchi and Harris (2007) reported for the observations done in natural light conditions. The antixenosis in the resistant line, 'Key 10', used in this study, is suggested as a major resistance component affecting both oviposition behavior and number of eggs laid.

Table 5.1. Treatments used in the experiment exploring the effect of spatial distribution of wheat spikes in the laboratory on oviposition rate of *Sitodiplosis mosellana*. The diagrams show the distribution of spikes inside laboratory cages.

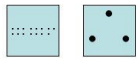
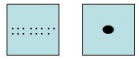
Test No.	Treatments	Replication	Flag leaf present	No. of female midges per arena	Spike distribution
No-choice tests					
1	Uniformly spaced vs. three 5-spike groups	6	Yes	15	
2	Uniformly spaced vs. clumped	6	Yes	15	
Choice tests					
3	Uniformly spaced vs. clumped	5	Yes	30	
4	Uniformly spaced vs. clumped	5	No	30	
5	Uniformly spaced vs. clumped	5	Yes	15	

Table 5.2. Results of reduced models after polynomial analysis of three variables from the behavioral observation of *Sitodiplosis mosellana* oviposition. Compound Symmetry covariance structure was applied in all of the analyses. Time interval was considered as a continuous variable. Differences are significant at $P < 0.05$.

	Duration of inter-oviposition interval		Number of eggs in 5-minute consecutive time intervals		Time for laying one egg	
Model effects	*F* value	*P*	*F* value	*P*	*F* value	*P*
Time interval*	$F_{1,318}$=36.40	<0.0001	$F_{1,196}$=7.82	0.0057	$F_{1,364}$=0.51	0.4775
Wheat line**	$F_{1,44}$=1.10	0.2990	$F_{1,47}$=5.07	0.0291	$F_{1,47}$=0.24	0.6293
Time interval×Wheat line	$F_{1,318}$=0.57	0.4504	$F_{1,196}$=0.36	0.5471	$F_{1,364}$=1.38	0.2408

* Refers to 5-min consecutive time intervals.

** Refers to 'Roblin' and 'Key 10'.

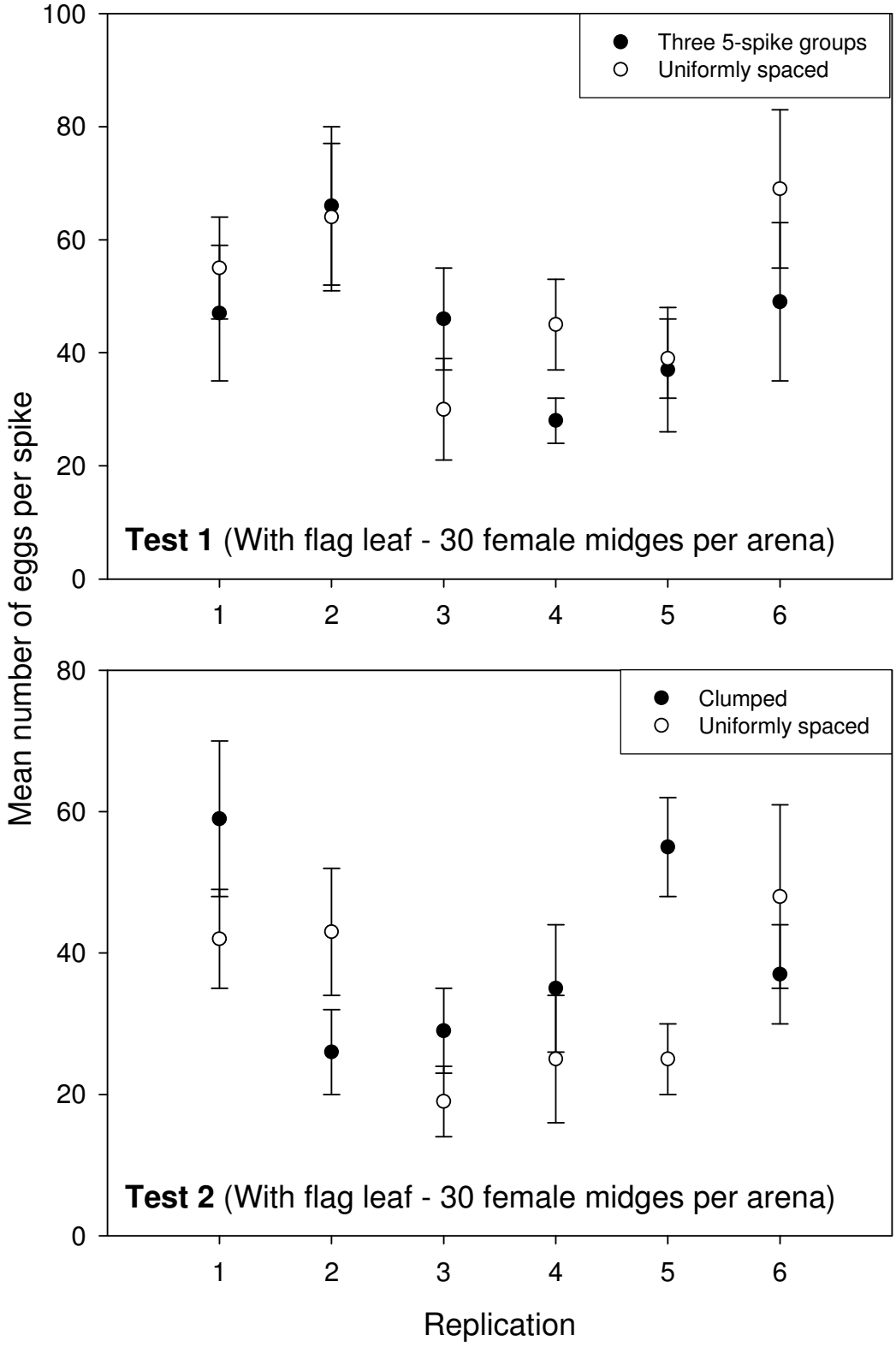


Figure 5.1. Mean (±SE) number of eggs per wheat spike in no-choice tests of the experiment exploring the effects of spatial distribution of wheat spikes on oviposition rate of *Sitodiplosis mosellana* in the laboratory.

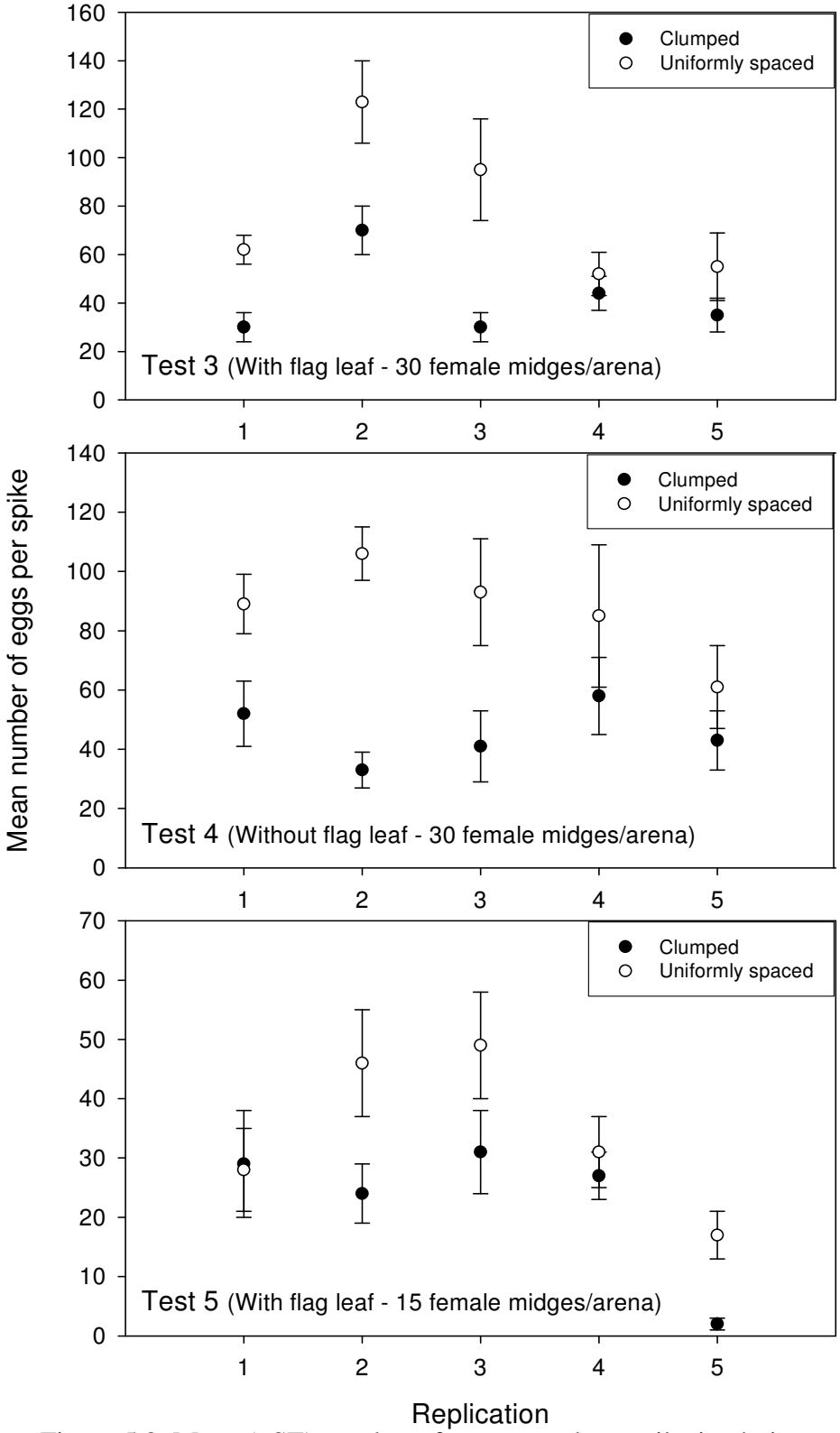


Figure 5.2. Mean (±SE) number of eggs per wheat spike in choice tests of the experiment exploring the effects of spatial distribution of wheat spikes on oviposition rate of *Sitodiplosis mosellana* in the laboratory.

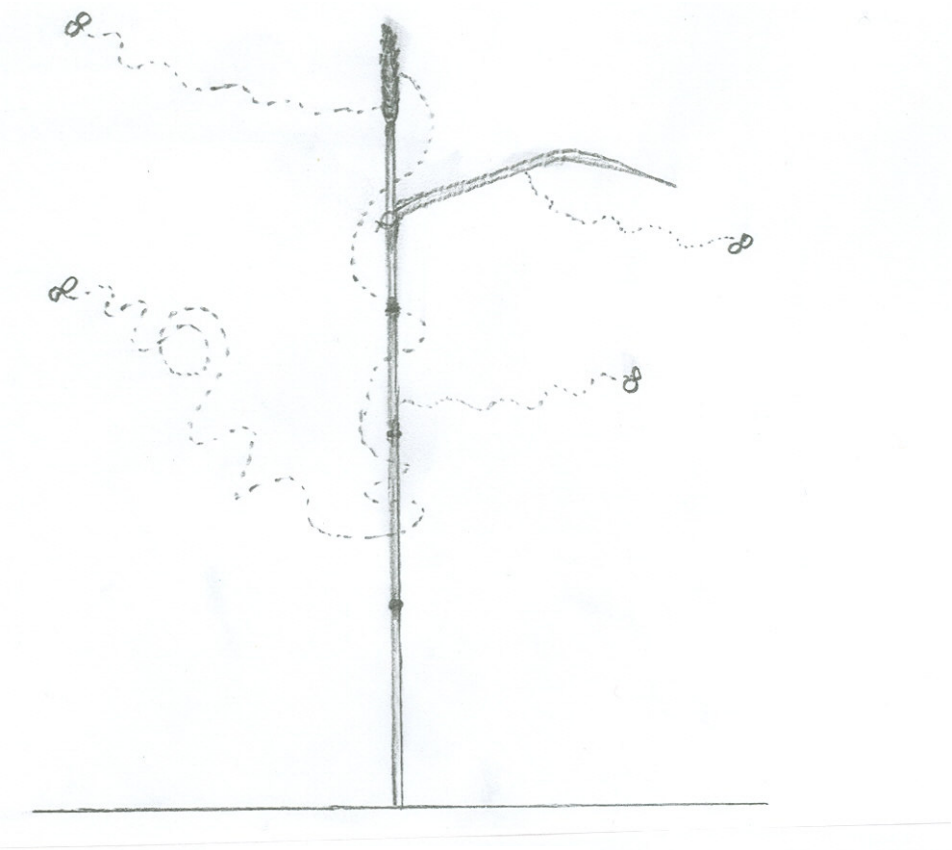


Figure 5.3. Different ways that female *Sitodiplosis mosellana* approached a bread wheat spike in the laboratory.

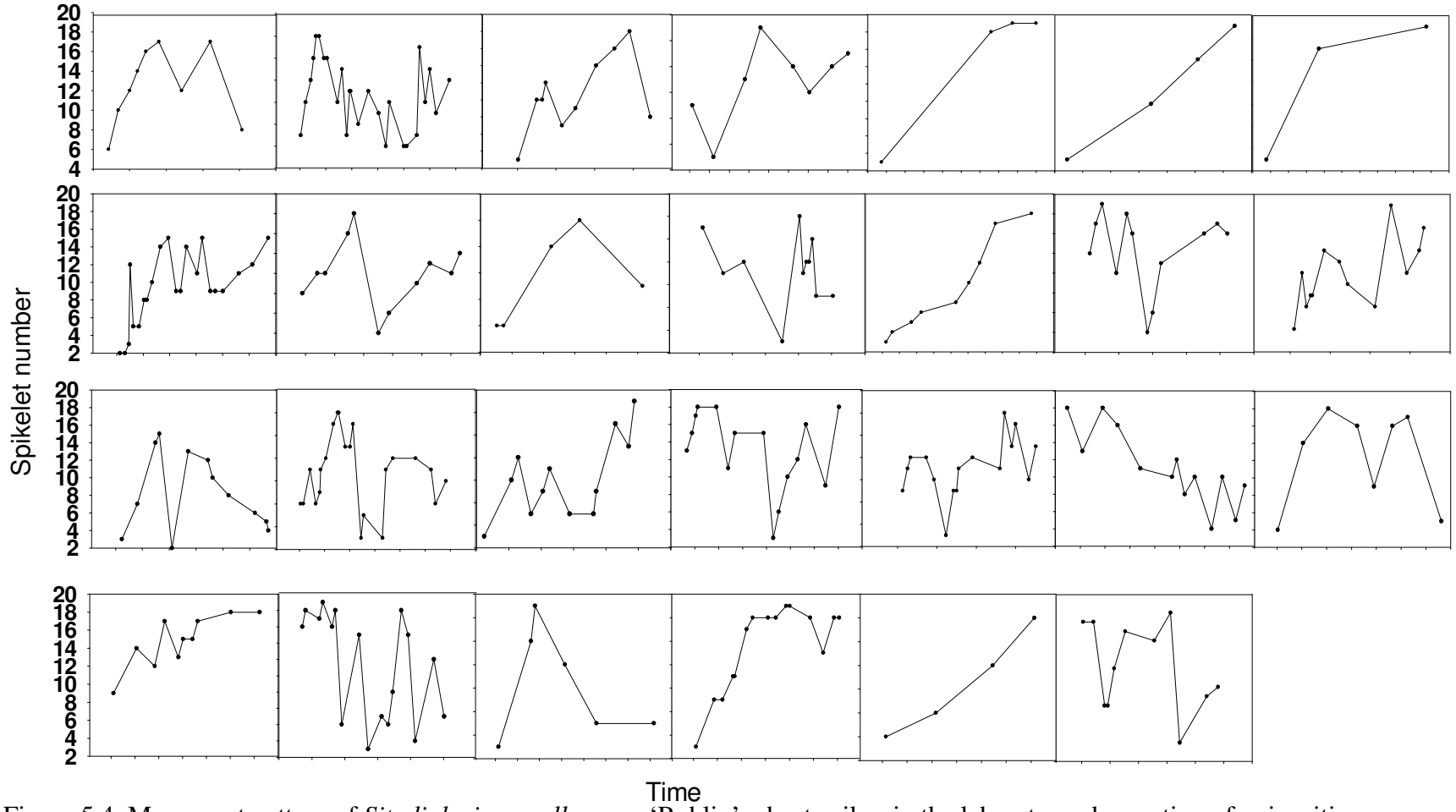


Figure 5.4. Movement pattern of *Sitodiplosis mosellana* on 'Roblin' wheat spikes in the laboratory observation of oviposition behavior. Each graph represents observation of one female. Each dot on the graph represents an oviposition event. The time axes are not necessarily equal, and illustrate only the passage time.

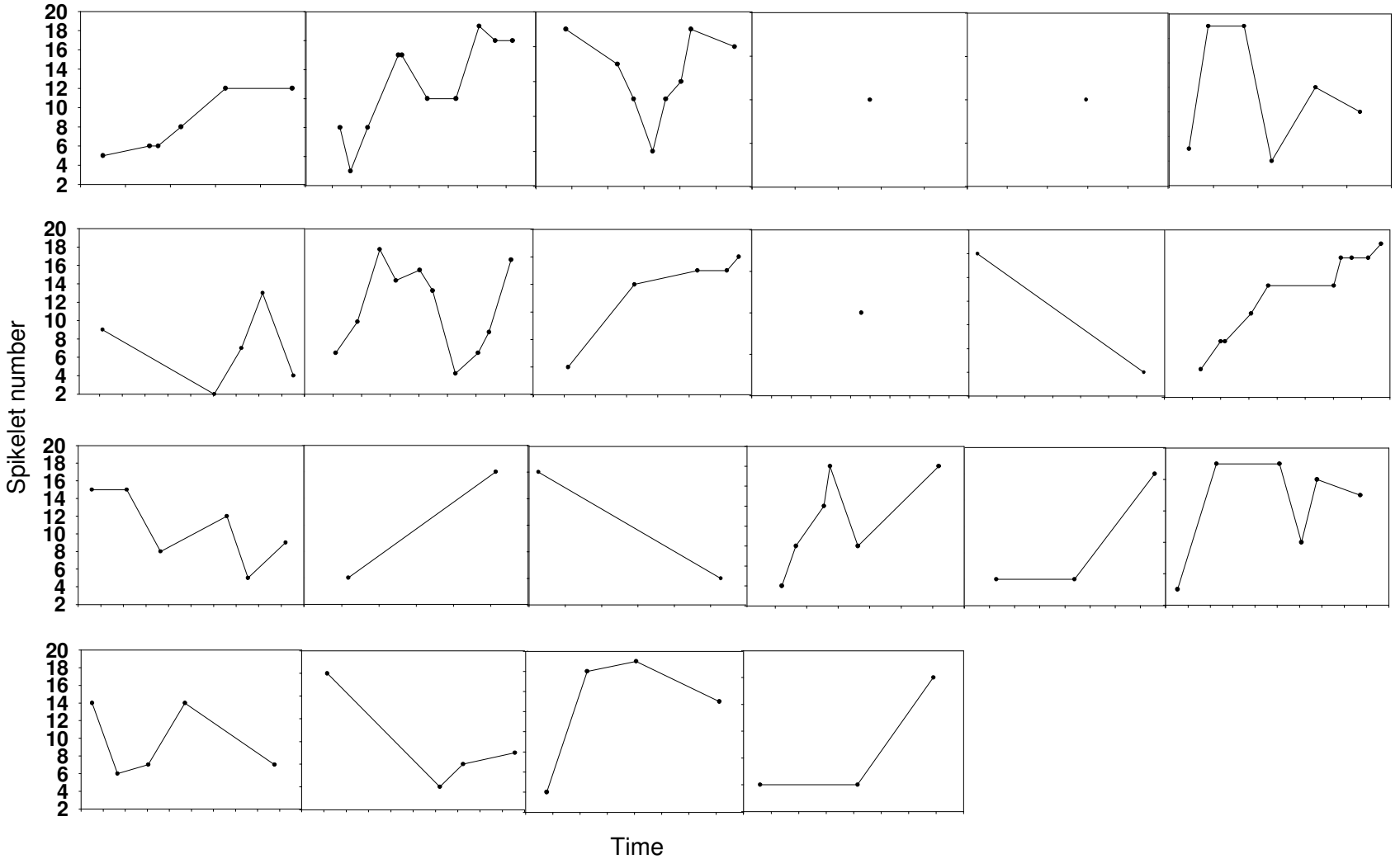


Figure 5.5. Movement pattern of *Sitodiplosis mosellana* on 'Key 10' wheat spikes in the laboratory observation of oviposition behavior. Each graph represents observation of one female. Each dot on the graph represents an oviposition event. The time axes are not necessarily equal, and illustrate only the passage time.

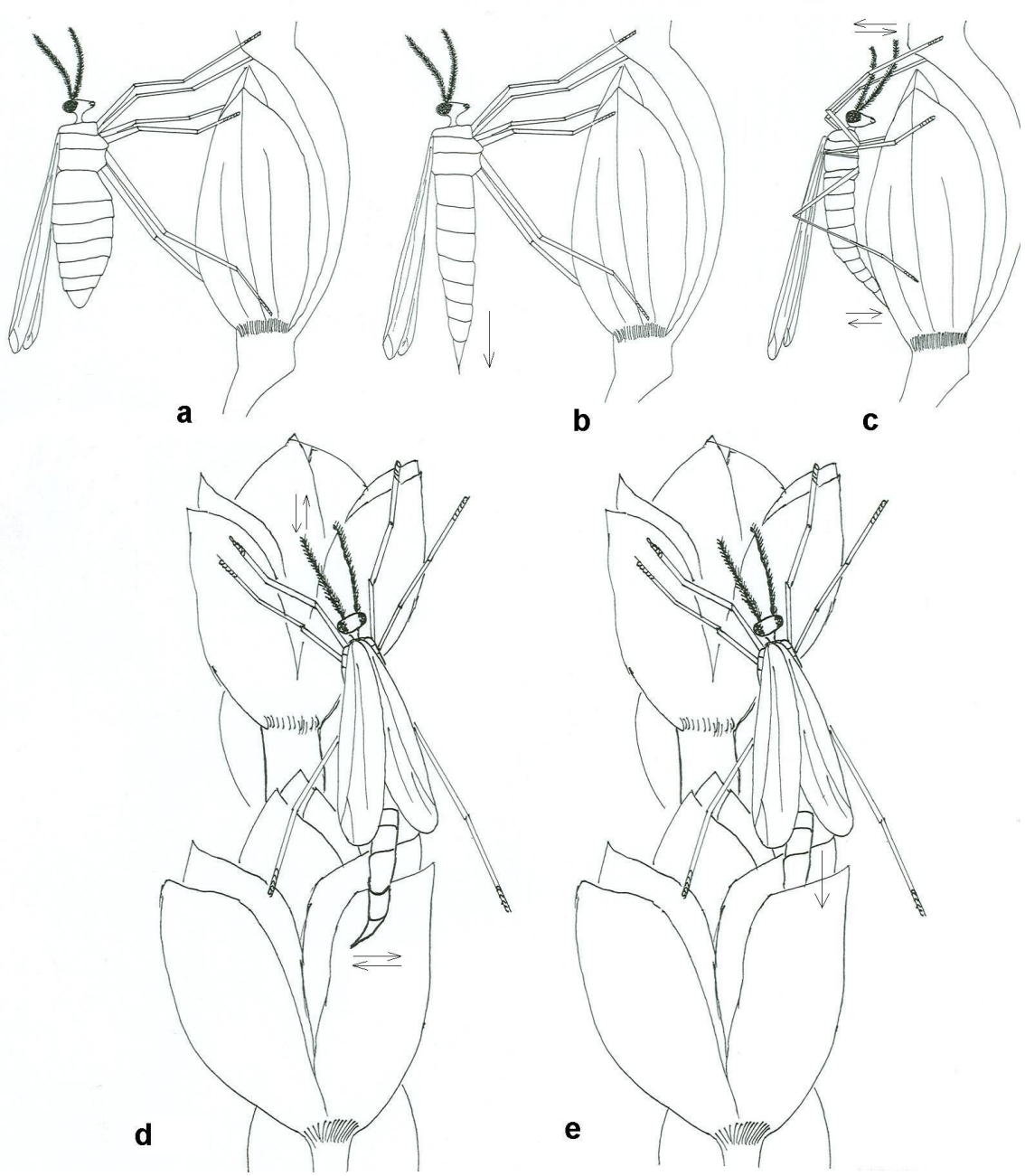


Figure 5.6. Schematic diagrams of oviposition behavior of *Sitodiplosis mosellana* on bread wheat spike in the laboratory. See text for details.
N.B.: The size of the insect is not proportional to the size of the plant. The insect was drawn at a larger scale to better show the details of the behavior.

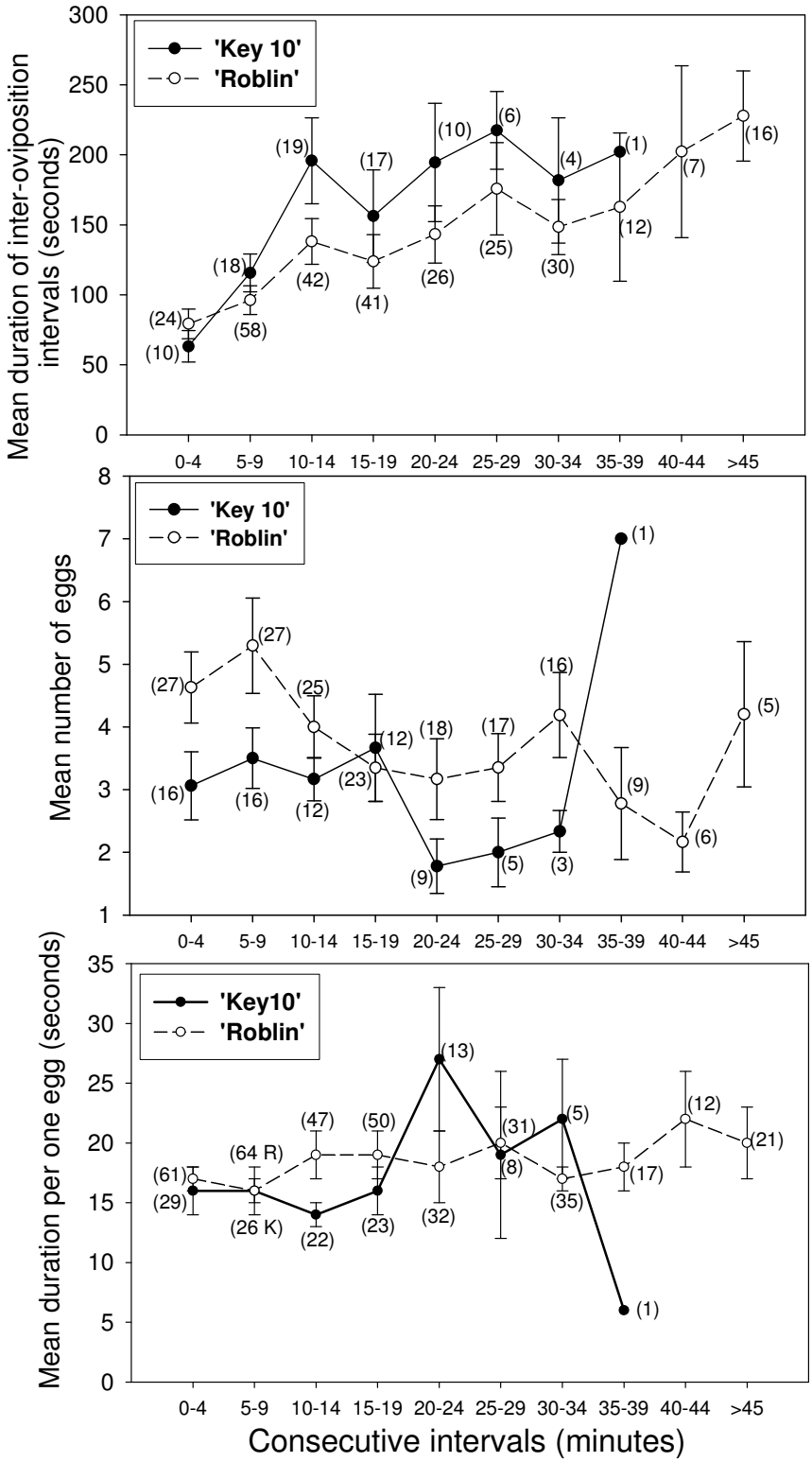


Figure 5.7. Mean duration of inter-oviposition intervals, mean number of eggs laid, and mean duration for laying one egg during consecutive 5-minute time intervals of on-spike activity on 'Roblin' and 'Key 10', by *Sitodiplosis mosellana*. Numbers in parentheses show the sample size.

Chapter 6

Summary and Conclusion

Wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), is a key pest of wheat in the Canadian Prairies. Deployment of wheat lines antixenotic against this pest may suppress oviposition in wheat fields and decrease yield loss. Antixenosis could also be used in conjunction with antibiosis to protect the antibiosis from the evolution of virulence. Our study was intended to clarify some of the interactions between wheat midge and wheat with emphasis on oviposition behavior. Moreover, we explored some genetic aspects of antixenosis in spring wheat lines. Our results can be a base for future exploration of antixenosis and may help breeders to develop lines resistant to wheat midge.

The effect of the background color behind wheat spikes in the laboratory on oviposition rate of wheat midge was studied. Black and red backgrounds decreased egg density on wheat spikes, and yellow and blue backgrounds increased egg density when used with spikes with red background in a laboratory choice test. The low egg density on wheat spikes with green background compared to spikes with yellow and blue backgrounds may indicate that low color contrast between an object and the background decreases oviposition rate of wheat midge. Our results revealed that wheat midge is affected by background color of wheat spikes and may use these visual cues for orienting toward the spikes of host plants.

In addition to exploring the effect of visual cues, we explored the effect of chemical cues of resistant and susceptible wheat spikes on wheat midge oviposition rate in the laboratory to determine if volatiles from host plants may have an effect on oviposition rate of wheat midge. The results indicated that wheat midge oviposition was affected by wheat spike volatiles. Volatiles of spikes of pre-anthesis 'Key 10', which is an antixenotic and also antibiotic wheat, suppressed oviposition of wheat midge, when the volatiles were added to pre-anthesis spikes of a susceptible wheat, 'Roblin'. A similar suppressing effect was observed when the volatiles of post-anthesis spikes of 'Roblin' were tested, consistent with the known reduction in oviposition on post-anthesis 'Roblin'. It is concluded that the wheat midge detects wheat spike volatiles, and one of the components of antixenosis of wheat lines to wheat midge might be the deterrent volatiles produced by spikes of antixenotic wheat lines. Our results did not provide evidence for presence of stimulatory compounds in wheat spikes, and provided evidence that low egg density on antixenotic wheat was not due to a lack of stimulants.

Details of the oviposition behavior of wheat midge on susceptible and resistant wheat in a laboratory study revealed some differences which provided evidence for considering antixenosis to be an effective and promising mechanism for developing lines resistant to this pest. Based on continuous observations of wheat midge oviposition behavior, antennation and ovipositor dragging on wheat spike surfaces are consistent behaviors on resistant or susceptible wheat. These behaviors suggest that female wheat midge detects the chemical cues of wheat spikes using her antennae and searches for tactile cues with her ovipositor. Duration for laying one egg did not differ on susceptible or resistant wheat, suggesting that duration for laying one egg depends on physiological

processes of the insect pushing the egg down the ovipositor and it might not be affected by cues received from wheat spikes. On susceptible wheat, wheat midge probed for a shorter time before laying the first egg compared to a resistant line. This might be due to the antixenotic factors of the resistant line such as deterrent volatiles produced by the spike. Wheat midge on-spike activity was longer on susceptible wheat, and oviposition rate was higher compared to resistant wheat. Wheat midges left spikes with a shorter delay after the last oviposition on susceptible wheat compared to a resistant line.

In order to better understand the allocation of wheat midge eggs among wheat spikes and to improve screening techniques for antixenosis detection in the laboratory, the effect of wheat spike distribution in the laboratory on wheat midge oviposition rate was explored. This revealed that all spikes in a confined arena are exploited by wheat midge regardless of their distribution, clumped or evenly distributed. However, the low egg density on the clumped wheat spikes compared to uniformly distributed spikes in a confined arena led to the conclusion that after oviposition, wheat midge flies over nearby spikes before searching for a new oviposition site. This behavior may have evolved to disperse unisexual progeny and increase mating success of progeny, or it might have evolved to decrease the impact of natural enemies which often show aggregative behavior or focussed local searching after a prey is discovered.

The above mentioned facts revealed that there are interactions between wheat midge and wheat, and antixenosis was significantly suppressing wheat midge oviposition. Wheat midge may use these cues to orient toward the host plants and choose suitable oviposition sites. The egg allocation pattern on a wheat spikelet might be also affected by interaction between chemical and tactile cues, as there were more eggs on the rachis of

the resistant lines compared to glume and floret. The higher egg densities on the upper and middle one-third sections of the wheat spikes compared to the lower one-third section might be due to cues produced by wheat plants related to the developmental pattern of wheat spikes.

We explored some genetic aspects of antixenosis from a crop breeding point of view, such as how may genes confer antixenosis and how antixenosis is inherited. Screening data for detection of antixenosis against wheat midge conducted in the laboratory and in two field-seasons showed differences among the 92 screened lines which were related doubled-haploid spring wheat lines from a single cross. There were 25 wheat lines which were consistently resistant in the field and laboratory, and these lines are assumed to have all the genes conferring antixenosis. There are probably two antixenosis genes in the screened lines, as the ratio of 67:25 lines fits a 3:1 ratio. We found that there was complementary interaction among genes, and the heritability of the antixenosis is 67%. Complementary interaction among antixenosis genes may indicate that the products of both genes are necessary for a wheat plant to express a high level of antixenosis against wheat midge. Our data did not provide evidence for linkage between the antibiosis gene, *Sm1*, and the genes conferring antixenosis. However, the fact that more than one gene interacts to confer antixenosis makes it difficult to determine the linkage between the antibiosis gene and individual antixenosis genes. We suggest that these antixenosis genes might be responsible for the production of deterrent volatiles by the wheat spikes. More research would clarify the location of genes which may be helpful in production of cultivars with antixenotic resistance to wheat midge. We tried to identify morphological traits of wheat spikes which might be inherited along with antixenosis and

might be used as phenotypic markers by breeders to facilitate screening lines for antixenosis to wheat midge. However, the studied traits were not sufficiently correlated to be considered as promising markers. In conclusion, the antixenosis described in this study promises to be a useful mechanism for suppressing wheat midge oviposition in the field and decreasing its damage.

Recommendations for Future Research

This study can be the basis for further exploration of the interactions between wheat midge and its host plants. We found that wheat midge reacts to visual, chemical and probably tactile cues. Evidence for the genetic basis of antixenosis to wheat midge in doubled-haploid wheat lines is provided. Future studies may be continued concentrating on the following questions:

1 - What are the chemical structures of the volatiles emitted by wheat spikes suppressing oviposition of wheat midge? The information would be helpful in finding which genes that control production of these volatiles.

2 - Where in the wheat genome are the genes that control antixenosis? Mapping the antixenosis genes could reveal if the antixenosis genes are on the same chromosome, or on different chromosomes, respective to the antibiosis gene, *Sm1,* or any gene producing undesirable traits. The results would be useful for crop breeders, determining the difficulty of transferring the antixenosis genes to commercial cultivars.

3 - Studies on other insects and cecidomyiids revealed that there were interactions among the cues used by an insect for locating and choosing a suitable host plant. Are there interactions among visual, chemical and tactile cues, regarding their effect on the oviposition of wheat midge on a wheat spikes? Obtaining more information about the cues used by an insect, and the interaction among the cues may reveal if one or more plant traits could be changed to obtain a plant cultivar resistant to the pest.

4 - Are there tactile cues, such as fine scale structure of wheat spike, affecting oviposition of wheat midge? The tactile cues might be manipulated in production of lines resistant to a pest.

5 - Are there stimulatory volatiles in the host plants susceptible to wheat midge, that make wheat midge oviposit more on susceptible host plants? If this is the case, the genes responsible for the stimulants might be excluded, to decrease oviposition rate on wheat.

6 - What is the effect of antixenosis in wheat lines on the natural enemies of wheat midge in the field? Factors conferring antixenosis, such as chemical or morphological traits of plant, might have deleterious or synergistic effects on the natural enemies of wheat midge. Exploring parasitism rates of eggs of wheat midge on the antixenotic lines in the field may be helpful for testing this hypothesis.

7 - Does wheat midge avoid oviposition on host plants which already contain conspecific eggs? The observation of oviposition behavior of wheat midge on host plants which already contain wheat midge eggs might be helpful to address this hypothesis.

8 - What is the effect of temperature on antixenosis genes and wheat midge oviposition behavior? Other research showed that by increasing the temperature, the resistance to some pests was lost in plants and plants became more susceptible. If the antixenotic wheat lines will be seeded in locations with high temperature, it would be useful to explore the effect of temperature on antixenosis genes and also wheat midge oviposition behavior.

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Appendix 1. Series number for the doubled-haploid spring wheat lines and their parents, 'Roblin' and 'Key 10', screened for antixenosis against *Sitodiplosis mosellana* in the field and laboratory.

Series	Name	Series	Name	Series	Name
501	'Roblin'	545	BD140*C43	589	BD140*C119
502	'Key10'	546	BD140*C52	590	BD140*C125
503	BD140*A36	547	BD140*C59	591	BD140*C136
504	BD140*A30	548	BD140*C60	592	BD140*C138
505	BD140*A12	549	BD140*C62	593	BD140*C139
506	BD140*A17	550	BD140*C67	594	BD140*C146
507	BD140*A25	551	BD140*C68		
508	BD140*A29	552	BD140*C88		
509	BD140*A1	553	BD140*D3		
510	BD140*A39	554	BD140*D8		
511	BD140*A42	555	BD140*D10		
512	BD140*A47	556	BD140*D20		
513	BD140*A55	557	BD140*D22		
514	BD140*A67	558	BD140*D24		
515	BD140*A84	559	BD140*D30		
516	BD140*A88	560	BD140*D41		
517	BD140*A95	561	BD140*D42		
518	BD140*A96	562	BD140*D53		
519	BD140*A101	563	BD140*D71		
520	BD140*A116	564	BD140*D72		
521	BD140*B7	565	BD140*D80		
522	BD140*B30	566	BD140*D84		
523	BD140*B38	567	BD140*D86		
524	BD140*B39	568	BD140*D86		
525	BD140*B58	569	BD140*D91		
526	BD140*B66	570	BD140*D102		
527	BD140*B68	571	BD140*D103		
528	BD140*B73	572	BD140*C89		
529	BD140*B80	573	BD140*C92		
530	BD140*B83	574	BD140*C93		
531	BD140*B87	575	BD140*C94		
532	BD140*B92	576	BD140*C95		
533	BD140*B108	577	BD140*C98		
534	BD140*B113	578	BD140*C103		
535	BD140*B114	579	BD140*C109		
536	BD140*B116	580	BD140*C110		
537	BD140*C13	581	BD140*C112		
538	BD140*C15	582	BD140*C113		
539	BD140*C19	583	BD140*C118		
540	BD140*C20	584	BD140*C127		
541	BD140*C28	585	BD140*C131		
542	BD140*C30	586	BD140*C135		
543	BD140*C33	587	BD140*C112		
544	BD140*C35	588	BD140*C117		

Appendix2.a. Number of eggs of wheat midge on doubled-haploid spring wheat lines in the field.

	Field 2006			Field 2007		
		Dunnett *P*			Dunnett *P*	
Line No.	Eggs/spike on test line	'Roblin'	'Key 10'	Eggs/spike on test line	'Roblin'	'Key 10'
501	16±3	-	0.385	146±9	-	<.0001
502	9±2	0.3847	-	30±3	<.0001	-
503	3±1	<.0001	0.092	44±7	<.0001	0.9998
504	15±2	1	0.618	49±6	<.0001	0.1919
505	17±4	1	0.861	45±5	<.0001	0.5812
506	15±4	1	0.998	31±4	<.0001	1
507	7±1	0.3061	1	-	-	-
508	6±2	0.3265	1	55±6	<.0001	0.0183
509	5±1	<.0001	0.304	37±4	<.0001	1
510	-	-	-	42±4	<.0001	0.8692
511	5±1	0.0018	0.906	18±2	<.0001	0.3968
512	4±1	<.0001	0.203	41±4	<.0001	0.8784
513	-	-	-	81±12	0.0008	<.0001
514	5±1	0.0018	0.909	29±6	<.0001	1
515	12±2	1	0.993	41±4	<.0001	0.8818
516	4±1	<.0001	0.146	25±4	<.0001	0.9393
517	6±1	0.0744	1	49±5	<.0001	0.1898
518	8±1	0.1962	1	55±4	<.0001	0.0157
519	-	-	-	59±5	<.0001	0.0043
520	4±1	<.0001	0.166	21±2	<.0001	0.9969
521	6±1	0.0054	0.991	68±5	<.0001	<.0001
522	6±1	0.0013	0.837	64±8	<.0001	0.001
523	3±1	<.0001	0.028	38±5	<.0001	1
524	4±1	0.0005	0.703	56±5	<.0001	0.0138
525	11±2	0.9712	1	25±4	<.0001	1

Continued.

Appendix 2.a. Continued.

	Field 2006			Field 2007		
		Dunnett *P*			Dunnett *P*	
Line No.	Eggs/spike on test line	'Roblin'	'Key 10'	Eggs/spike on test line	'Roblin'	'Key 10'
526	8±1	0.5197	1	92±11	0.004	<.0001
527	8±2	0.0695	1	57±4	<.0001	0.0053
528	3±1	<.0001	0.027	34±2	<.0001	1
529	7±2	0.0806	1	74±6	<.0001	<.0001
530	9±3	0.0522	1	34±10	<.0001	1
531	24±15	1	0.988	73±5	<.0001	<.0001
532	12±2	0.9999	1	64±8	<.0001	0.0023
533	10±1	0.8695	1	72±8	<.0001	<.0001
534	10±2	0.7601	1	57±3	<.0001	0.0034
535	7±1	0.211	1	69±8	<.0001	0.0002
536	5±1	0.0008	0.786	20±3	<.0001	0.4838
537	2±2	0.0005	0.661	37±3	<.0001	0.9985
538	3±1	<.0001	0.139	33±4	<.0001	1
539	4±1	0.0003	0.584	48±5	<.0001	0.2163
540	2±1	<.0001	0.002	-	-	-
541	9±2	0.2622	1	43±6	<.0001	0.8678
542	5±1	0.0033	0.969	38±5	<.0001	1
543	-	-	-	44±4	<.0001	0.4257
544	-	-	-	40±5	<.0001	1
545	5±1	0.0002	0.497	18±2	<.0001	0.5593
546	6±1	0.0011	0.826	21±4	<.0001	0.3763
547	6±1	0.0141	1	65±7	<.0001	0.0004
548	10±3	0.2146	1	72±8	<.0001	<.0001
549	9±1	0.5155	1	101±11	0.054	<.0001
550	16±3	1	0.783	42±6	<.0001	0.994

Continued.

Appendix 2.a. Continued.

	Field 2006			Field 2007		
		Dunnett *P*			Dunnett *P*	
Line No.	Eggs/spike on test line	'Roblin'	'Key 10'	Eggs/spike on test line	'Roblin'	'Key 10'
551	12±2	1	0.999	53±4	<.0001	0.0363
552	7±1	0.2296	1	29±4	<.0001	1
553	13±3	1	1	61±6	<.0001	0.0021
554	4±1	<.0001	0.324	17±3	<.0001	0.0468
555	10±2	0.7023	1	27±3	<.0001	1
556	14±3	0.995	1	60±8	<.0001	0.035
557	9±1	0.8415	1	46±3	<.0001	0.2792
558	11±2	0.9939	1	69±7	<.0001	<.0001
559	9±2	0.559	1	73±7	<.0001	<.0001
560	7±2	0.0379	1	30±4	<.0001	1
561	-	-	-	72±9	<.0001	0.0015
562	7±1	0.4208	1	31±4	<.0001	1
563	7±2	0.0171	1	33±4	<.0001	1
564	6±1	0.026	1	77±6	<.0001	<.0001
565	7±1	0.0964	1	41±4	<.0001	0.8326
566	14±2	1	0.93	61±5	<.0001	0.0011
567	4±1	0.0004	0.645	33±4	<.0001	1
568	3±1	<.0001	0.013	22±3	<.0001	0.9208
569	10±2	0.5104	1	80±9	<.0001	<.0001
570	15±2	1	0.616	38±3	<.0001	0.9953
571	11±2	0.9913	1	68±6	<.0001	<.0001
572	12±2	1	0.995	40±8	<.0001	1
573	10±2	1	1	99±7	0.081	<.0001
574	6±1	0.0076	0.998	29±4	<.0001	1
575	-	-	-	46±9	<.0001	0.9991

Continued.

Appendix 2.a. Continued.

	Field 2006			Field 2007		
		Dunnett *P*			Dunnett *P*	
Line No.	Eggs/spike on test line	'Roblin'	'Key 10'	Eggs/spike on test line	'Roblin'	'Key 10'
576	10±2	0.9793	1	60±4	<.0001	0.0011
577	8±1	0.3643	1	55±5	<.0001	0.0307
578	10±2	0.1326	1	68±9	<.0001	0.0008
579	4±1	<.0001	0.294	51±4	<.0001	0.0592
580	8±2	0.0302	1	54±7	<.0001	0.1152
581	9±2	0.2349	1	61±8	<.0001	0.0085
582	14±3	1	1	56±6	<.0001	0.027
583	-	-	-	32±5	<.0001	1
584	3±1	<.0001	0.01	54±6	<.0001	0.0446
585	-	-	-	-	-	-
586	6±1	0.0031	0.963	59±6	<.0001	0.0065
587	7±1	0.0519	1	90±7	0.006	<.0001
588	9±1	0.9991	1	33±4	<.0001	1
589	6±3	0.0018	0.655	-	-	-
590	8±1	0.2853	1	70±7	<.0001	<.0001
591	4±1	<.0001	0.1	32±3	<.0001	1
592	7±1	0.0068	0.996	72±10	<.0001	0.0003
593	10±2	0.8999	1	56±5	<.0001	0.0113
594	4±1	0.0001	0.267	20±4	<.0001	0.2294

Appendix 2.b. Number of eggs of wheat midge on doubled-haploid wheat lines in laboratory, and comparisons with parents.

Laboratory														
			‘Roblin’							‘Key 10’				
					Statistics							Statistics		
Line No.	No. of spikes tested	Egg/spike on test line	No. of spikes tested	Egg/spike on ‘Roblin’	Den.	$F_{1,Den.}$	*P*	No. of spikes tested	Egg/spike on test line	No. of spikes tested	Egg/spike on ‘Key 10’	Den. *	$F_{1,Den.}$	*P*
501	-	-	-	-	-	-	-	-	-	-	-	-	-	-
502	-	-	-	-	-	-	-	-	-	-	-	-	-	-
503	10	90±22	12	109±22	4.14	2.69	0.174	10	90±22	12	12±4	4.24	9.43	0.0344
504	10	24±5	16	140±15	24	61.64	<.0001	10	23±5	15	24±9	6.8	1.21	0.3095
505	10	46±8	12	96±22	10.8	2.01	0.1841	10	46±8	12	24±8	4.4	5.18	0.0791
506	8	34±20	8	167±39	5.94	7.97	0.0306	8	34±20	8	38±8	5.98	0.28	0.6181
507	18	44±8	20	228±28	9.49	51.11	<.0001	14	39±9	16	41±7	6.53	0.01	0.9182
508	14	62±13	12	126±26	5.36	2.98	0.1411	14	62±13	11	12±5	10.1	10.1	0.0099
509	9	29±14	9	157±36	5.01	15.01	0.0116	9	29±14	10	33±8	12.1	0.88	0.3661
510	-	-	-	-	-	-	-	-	-	-	-	-	-	-
511	10	46±10	10	212±32	7.66	26.78	0.001	10	46±10	10	29±7	7.16	2	0.199
512	10	34±9	9	124±19	4.46	33.3	0.0032	10	34±9	10	30±11	14.9	0.91	0.3566
511	13	139±42	12	234±40	5.25	5.59	0.0619	13	139±42	12	31±7	18.1	14.9	0.0011
514	10	48±22	14	125±27	6.98	15.26	0.0059	10	48±22	14	37±13	16.1	0.69	0.4193
515	10	63±14	14	138±28	16.2	12.4	0.0028	10	63±14	14	28±8	5.78	3.13	0.1292
516	18	19±6	20	215±26	9.3	95.88	<.0001	14	24±7	15	43±7	5.84	3.3	0.1208
517	10	55±10	14	108±21	7.04	9.49	0.0177	10	55±10	14	10±3	4.7	35.1	0.0024
518	11	50±10	14	115±28	6.81	5.67	0.0498	11	50±10	14	28±8	18.3	5.45	0.0312
519	-	-	-	-	-	-	-	-	-	-	-	-	-	-
520	14	30±7	14	112±18	20.2	29.51	<.0001	14	30±7	14	39±8	20.2	0.58	0.4553
521	15	101±19	14	97±16	21.3	0.05	0.8309	15	101±19	14	27±9	5.17	27.6	0.003
522	10	49±12	14	154±26	6.73	24.82	0.0018	10	49±12	14	47±12	5.99	0.15	0.7105
523	11	24±7	14	158±29	17.6	39.94	<.0001	11	24±7	14	28±10	17	0.94	0.345
524	17	70±13	20	151±19	26	21.69	<.0001	15	70±15	17	37±12	7.45	7.49	0.0274
525	10	29±6	10	103±39	3.9	6.7	0.0622	10	29±6	10	14±6	18	6.16	0.0231
526	15	88±18	16	109±20	6.1	0.32	0.592	15	88±18	16	14±5	22	84.3	<.0001
527	9	82±19	6	67±19	11.8	0.25	0.629	9	82±19	6	5±2	3.42	23	0.0127
528	24	46±6	20	187±23	33.4	112	<.0001	20	47±7	16	58±10	5.68	0.15	0.7149
529	16	52±7	12	138±41	4.9	2.18	0.2013	16	52±7	12	34±13	20.8	12.2	0.0022
530	11	42±9	12	221±36	16.1	63.8	<.0001	7	56±9	8	47±11	3.16	0.39	0.5765

* Denominator

Continued.

Appendix 2.b. Continued.

Laboratory														
			'Roblin'							'Key 10'				
					Statistics							Statistics		
Line No.	No. of spikes tested	Egg/spike on test line	No. of spikes tested	Egg/spike on 'Roblin'	Den.	$F_{1,Den.}$	*P*	No. of spikes tested	Egg/spike on test line	No. of spikes tested	Egg/spike on 'Key 10'	Den.*	$F_{1,Den.}$	*P*
531	10	130±38	8	125±36	5.83	0.22	0.6542	10	130±38	8	19±6	2.64	4.66	0.1319
532	10	80±13	10	198±28	14.2	25.03	0.0002	10	80±13	10	53±15	4.15	1.39	0.3012
533	11	58±16	12	96±12	5.1	3.97	0.1018	11	58±16	12	16±4	10.2	6.41	0.0294
534	12	59±11	12	191±30	17	51.14	<.0001	12	59±11	12	30±6	17	5.94	0.026
535	8	106±19	10	135±20	16	0.82	0.3793	6	103±20	7	25±10	3.57	21.1	0.0131
536	8	48±12	10	114±22	11.1	13.74	0.0034	8	48±12	9	31±13	2.12	4.27	0.1675
537	12	37±8	14	172±26	5.91	38.55	0.0009	12	37±8	14	50±8	17.8	1.87	0.1885
538	10	24±9	12	118±22	15.2	26.48	0.0001	10	24±9	12	18±10	15	1.69	0.2135
539	23	42±8	18	160±22	8.18	28.53	0.0006	21	46±9	16	60±9	6.4	3.29	0.1166
540	-	-	-	-	-	-	-	-	-	-	-	-	-	-
541	-	-	-	-	-	-	-	-	-	-	-	-	-	-
542	11	49±11	12	209±36	16.2	58.01	<.0001	11	49±11	12	31±6	16.7	3.49	0.0793
543	10	41±9	10	230±41	13.6	33.97	<.0001	10	41±9	10	34±6	17.2	0.19	0.6648
544	10	45±10	8	146±47	13.5	6.65	0.0224	10	45±10	8	14±7	16	8.69	0.0094
545	15	43±7	16	218±27	22.2	59.84	<.0001	11	48±8	12	47±8	4.87	0.08	0.7882
546	16	19±8	16	199±26	23.5	102.1	<.0001	14	13±6	13	35±7	6.26	7.13	0.0355
547	10	41±9	8	232±56	5.84	8.83	0.0258	10	41±9	8	33±9	5.84	0.12	0.7418
548	10	76±16	12	174±10	6.44	21.41	0.003	10	76±16	12	49±9	6.22	3.85	0.0956
549	11	106±19	8	178±46	6.9	2.4	0.166	11	106±7	8	16±6	15.3	17.3	0.0008
550	10	60±7	8	109±23	14.8	4.33	0.0552	10	60±7	8	26±6	14.3	14	0.0022
551	15	16±5	14	164±26	6.57	85.35	<.0001	15	16±5	13	27±8	6.02	0.63	0.4558
552	9	14±6	5	118±36	1.31	13.52	0.1216	9	14±6	6	11±6	13	0.12	0.7372
553	14	143±18	14	142±31	20.1	0	0.9594	14	143±18	14	14±5	20.2	63.2	<.0001
554	14	39±10	14	92±17	5.85	7.36	0.0359	14	39±10	14	26±9	20.3	0.98	0.3332
555	22	17±4	16	162±23	7.83	117.8	<.0001	22	17±4	15	53±10	28	31	<.0001
556	11	45±10	10	205±45	3.87	13.83	0.0217	11	45±10	10	35±11	3.87	0.63	0.4726
557	10	33±8	10	168±38	4.19	22.79	0.0078	10	33±8	10	28±8	13.9	0.83	0.3778
558	10	49±15	9	97±16	3.9	7.51	0.0533	10	49±15	10	25±8	4	2.82	0.1684
559	10	28±12	7	133±26	11.9	23.64	0.0004	10	28±12	7	26±11	12.3	0.96	0.3473
560	11	13±4	12	218±33	16.1	109.3	<.0001	7	18±5	8	54±11	9.43	3.56	0.0905

* Denominator

Continued.

Appendix 2.b. Continued.

Laboratory														
			'Roblin'							'Key 10'				
					Statistics							Statistics		
Line No.	No. of spikes tested	Egg/spike on test line	No. of spikes tested	Egg/spike on 'Roblin'	Den.	$F_{1,Den.}$	*P*	No. of spikes tested	Egg/spike on test line	No. of spikes tested	Egg/spike on 'Key 10'	Den*.	$F_{1,Den.}$	*P*
561	12	34±10	10	173±31	16.4	74.96	<.0001	12	34±10	10	45±18	4.29	0.01	0.9378
562	10	29±7	10	169±24	14.5	68.74	<.0001	10	29±7	9	25±9	8	0.56	0.4753
563	12	52±14	14	200±34	6.49	23.37	0.0023	8	73±17	9	46±10	11.3	2.01	0.1828
564	9	75±15	13	93±22	6.85	0.04	0.848	9	75±15	14	13±4	21	19.8	0.0002
565	12	64±13	12	227±38	17.5	25.09	<.0001	10	68±15	10	45±8	14.7	3.01	0.1038
566	8	59±10	10	124±22	12.4	6.93	0.0214	8	59±10	10	27±10	8.38	5.74	0.0422
567	25	36±6	20	177±23	34.3	91.66	<.0001	23	37±7	18	57±9	31.1	7.53	0.01
568	15	37±12	18	125±17	14.9	15.07	0.0015	15	37±12	18	24±9	7.76	1.88	0.2081
569	14	57±9	16	180±28	6.76	15.9	0.0057	12	59±11	14	63±11	5.5	0.17	0.6957
570	11	49±6	10	129±38	4.16	6.22	0.0647	11	49±6	10	16±6	18.2	13.4	0.0018
571	10	38±8	10	69±13	7.5	3.66	0.0945	10	38±8	10	20±4	14.9	3.95	0.0655
572	10	52±10	6	132±61	10.9	6.84	0.0242	10	52±10	6	14±9	10.6	10.5	0.0082
573	10	70±9	6	31±5	11.2	25.05	0.0004	10	70±9	6	11±6	2.18	35.9	0.0218
574	9	51±19	9	164±38	3.92	20.84	0.0108	9	51±19	10	59±17	13.3	0.06	0.8169
575	10	26±13	8	123±21	7.76	29	0.0007	10	26±13	8	27±15	13.7	0.02	0.895
576	10	96±14	4	33±7	12	12.9	0.0037	10	96±14	4	10±9	1.81	13.9	0.0757
577	17	55±11	16	115±29	22	12.12	0.0021	17	55±11	16	11±4	22.9	40.9	<.0001
578	9	86±27	10	138±21	5.1	8.69	0.0312	9	86±27	10	22±11	3.61	8	0.0534
579	10	75±14	4	81±12	1	0.76	0.5432	10	75±14	4	11±6	8.75	10.6	0.0103
580	10	27±4	10	155±18	5.92	56.42	0.0003	10	27±4	10	24±9	8.22	0.51	0.4952
581	11	91±15	6	252±49	3.09	12.5	0.0367	11	91±15	6	46±13	1.49	3.55	0.2415
582	10	37±12	4	78±20	11.8	3.23	0.0979	10	37±12	4	20±7	12	0.46	0.509
583	11	29±7	14	125±20	23	32.68	<.0001	11	29±7	14	9±3	5.94	8.48	0.0273
584	15	72±12	12	132±34	20.3	4.8	0.0403	15	72±12	12	21±4	8.58	12.7	0.0065
585	9	29±7	10	140±26	8.3	19.4	0.0021	9	29±7	10	11±5	8.15	3.21	0.1104
586	13	85±15	14	128±19	12.1	2.2	0.1637	13	85±15	14	15±4	6.3	49.1	0.0003
587	10	146±30	6	194±61	2.28	0.44	0.5699	10	146±30	6	23±8	1.99	11	0.0807
588	10	54±10	10	107±18	8	4.36	0.0701	10	54±10	10	10±4	14	35.8	<.0001
589	15	53±10	11	103±18	19.6	10.62	0.004	15	53±10	12	33±8	21	3.6	0.0717
590	10	22±6	9	101±15	13.3	59.08	<.0001	10	22±6	10	17±6	3.31	2.62	0.1952

* Denominator

Continued.

Appendix 2.b. Continued.

	Laboratory													
			‘Roblin’							‘Key 10’				
					Statistics							Statistics		
Line No.	No. of spikes tested	Egg/spike on test line	No. of spikes tested	Egg/spike on ‘Roblin’	Den.	$F_{1,Den.}$	*P*	No. of spikes tested	Egg/spike on test line	No. of spikes tested	Egg/spike on ‘Key 10’	Den*	$F_{1,Den.}$	*P*
591	10	23±6	6	136±24	11.6	36.99	<.0001	10	23±6	6	36±15	12.6	1.17	0.2996
592	10	36±11	5	181±66	4.91	8.66	0.0328	10	36±11	6	39±10	13.2	1.4	0.2583
593	10	36±10	4	78±20	5.24	7.64	0.0377	10	36±10	4	20±7	12	0.86	0.3729
594	9	38±7	8	80±15	15	6.13	0.0257	9	38±7	8	21±5	11.6	3.45	0.0888

* Denominator

Appendix 3. Wheat midge larval densities on doubled-haploid spring wheat lines in the field.

	Field 2006					Field 2007				
Line No.	No. of spikes	No. of dead larvae	No. of living larvae	No. of damaged seeds	*Sm1* present	No. of spikes	No. of dead larvae	No. of living larvae	No. of damaged seeds	*Sm1* present
503	4	2	0	1	yes	3	8	0	0	yes
504	1	1	1	2	yes	4	7	0	2	yes
505	1	0	8	5	no	-	-	-	-	-
506	-	-	-	-	-	8	23	4	27	yes
507	2	6	8	9	no	-	-	-	-	-
508	-	-	-	-	-	3	0	0	1	yes
509	5	0	0	1	yes	5	13	4	8	yes
510	-	-	-	-	-	-	-	-	-	-
511	-	-	-	-	-	6	0	15	12	no
512	1	0	3	3	no	3	1	15	8	no
513	-	-	-	-	-	-	-	-	-	-
514	2	0	6	6	no	1	0	4	4	no
515	1	0	35	13	no	2	0	22	11	no
516	3	0	0	3	yes	2	0	0	0	-
517	2	0	11	5	no	9	1	81	37	no
518	1	3	1	2	yes	4	5	1	8	yes
519	-	-	-	-	-	2	1	36	18	no
520	3	0	9	7	no	3	0	4	1	no
521	1	4	0	3	yes	4	8	11	17	no
522	1	1	0	2	yes	4	11	10	9	yes
523	1	0	0	2	no	7	3	0	8	yes
524	2	0	10	9	no	1	0	9	5	no
525	-	-	-	-	-	-	-	-	-	-
526	1	1	0	3	yes	5	3	7	7	no
527	3	0	0	5	yes	3	8	0	7	yes
528	1	0	11	6	no	5	0	37	20	no
529	3	5	0	6	yes	3	18	0	2	yes
530	-	-	-	-	-	1	0	12	3	no
531	2	4	0	6	yes	1	0	5	4	no

Continued.

Appendix 3. Continued.

	Field 2006					Field 2007				
Line No.	No. of spikes	No. of dead larvae	No. of living larvae	No. of damaged seeds	*Sm1* present	No. of spikes	No. of dead larvae	No. of living larvae	No. of damaged seeds	*Sm1* present
532	3	1	0	3	yes	2	10	0	3	yes
533	1	3	0	3	yes	5	9	3	9	yes
534	1	0	3	2	no	9	7	28	22	no
535	2	2	0	1	yes	1	0	4	3	no
536	6	2	0	4	yes	3	4	0	1	yes
537	5	0	14	9	no	5	6	25	14	no
538	-	-	-	-	-	4	3	18	11	no
539	1	1	0	1	yes	7	7	0	7	yes
540	5	7	0	2	yes	-	-	-	-	-
541	1	0	5	5	no	7	13	10	11	no
542	5	0	8	6	no	2	0	23	13	no
543	-	-	-	-	-	2	8	0	5	yes
544	-	-	-	-	-	-	-	-	-	-
545	1	0	1	1	no	6	4	66	26	no
546	1	0	5	4	no	1	0	0	0	-
547	1	0	13	6	no	3	0	53	20	no
548	3	2	0	2	yes	8	20	0	5	yes
549	3	5	0	3	yes	3	7	2	8	yes
550	-	-	-	-	-	5	3	2	4	yes
551	-	-	-	-	-	2	0	40	18	no
552	1	0	2	2	no	5	6	5	9	yes
553	1	1	1	2	yes	4	0	21	15	no
554	1	0	1	1	no	3	1	12	5	no
555	1	0	10	5	no	5	0	36	17	no
556	1	1	0	5	yes	3	6	0	7	yes
557	1	1	0	3	yes	5	14	3	12	yes
558	1	0	0	3	no	2	8	0	6	yes
559	1	0	21	10	no	2	1	29	13	no

Continued.

Appendix 3. Continued.

	Field 2006					Field 2007				
Line No.	No. of spikes	No. of dead larvae	No. of living larvae	No. of damaged seeds	*Sm1* present	No. of spikes	No. of dead larvae	No. of living larvae	No. of damaged seeds	*Sm1* present
560	-	-	-	-	-	1	0	7	3	no
561	-	-	-	-	-	2	2	13	8	no
562	1	0	0	2	yes	4	0	21	4	no
563	1	0	11	8	no	6	0	60	33	no
564	-	-	-	-	-	3	3	28	13	no
565	-	-	-	-	-	5	4	6	10	no
566	1	1	1	3	yes	6	25	2	32	yes
567	2	0	8	8	no	3	0	5	3	no
568	2	0	0	1	yes	6	5	0	6	yes
569	1	5	0	2	yes	5	0	0	0	-
570	-	-	-	-	-	4	14	4	10	yes
571	1	3	1	3	yes	8	39	4	37	yes
572	1	0	13	5	no	4	0	52	19	no
573	2	6	6	8	no	2	6	20	15	no
574	1	1	0	1	yes	6	7	1	5	yes
575	-	-	-	-	-	-	-	-	-	-
576	1	0	18	8	no	1	0	12	6	no
577	1	0	11	5	no	6	0	86	37	no
578	1	0	15	6	no	1	0	18	4	no
579	11	5	9	11	no	5	1	29	15	no
580	5	0	3	1	no	-	-	-	-	-
581	1	1	0	1	yes	4	3	0	2	yes
582	-	-	-	-	-	9	15	1	13	yes
583	-	-	-	-	-	1	0	0	0	-
584	1	2	0	5	yes	6	12	0	2	yes
585	-	-	-	-	-	1	1	0	0	-
586	2	0	9	5	no	2	0	17	9	no
587	2	0	10	6	no	7	0	139	53	no

Continued.

Appendix 3. Continued.

	Field 2006					Field 2007				
Line No.	No. of spikes	No. of dead larvae	No. of living larvae	No. of damaged seeds	*Sm1* present	No. of spikes	No. of dead larvae	No. of living larvae	No. of damaged seeds	*Sm1* present
588	-	-	-	-	-	-	-	-	-	-
589	-	-	-	-	-	-	-	-	-	-
590	4	5	0	3	yes	3	6	0	6	yes
591	1	0	11	3	no	5	2	39	17	no
592	1	0	2	1	no	3	13	1	10	yes
593	1	1	0	4	yes	3	2	0	3	yes
594	1	0	0	1	yes	3	5	0	5	yes

Appendix 4. Spearman correlation (r_s) for relationships among morphological traits of spring wheat lines grown in the laboratory. The traits were measured at pre-anthesis stage. See Table 4.1 for description of the traits. Correlations are significant at $P < 0.05$.

Trait No.	3	5	6	7	8	10	11	13	14	15	16	18	19	20	21
1	0.2828	0.0036	0.0728	0.0733	0.0659	0.1901	0.0528	0.1320	0.0301	0.1061	0.0684	0.1394	0.1394	0.0847	0.0746
	<.0001	0.9200	0.0406	0.0391	0.0634	<.0001	0.1375	0.0002	0.3968	0.0028	0.0542	<.0001	<.0001	0.0171	0.0357
3		0.0759	0.2323	0.1707	0.0032	-0.106	0.2349	-0.075	0.2031	0.1684	0.0698	0.0304	0.0927	0.1873	0.0803
		0.0328	<.0001	<.0001	0.9276	0.0028	<.0001	0.0345	<.0001	<.0001	0.0498	0.3936	0.0092	<.0001	0.0240
5			-0.058	0.0295	0.0730	-0.0593	0.0322	-0.0039	-0.008	0.0334	-0.028	-0.134	0.0083	0.0028	0.0663
			0.0967	0.4023	0.0379	0.0922	0.3610	0.9127	0.8167	0.3428	0.4113	0.0001	0.8133	0.9364	0.0598
6				0.3363	0.0855	-0.104	0.4864	-0.045	0.4632	0.1718	-0.068	0.0099	0.0190	0.3031	0.1372
				<.0001	0.0152	0.0031	<.0001	0.1983	<.0001	<.0001	0.0534	0.7791	0.5910	<.0001	<.0001
7					0.0855	-0.153	0.3575	0.0195	0.3467	0.2493	0.0463	0.0347	0.0980	0.1703	0.3409
					0.0152	<.0001	<.0001	0.5812	<.0001	<.0001	0.1885	0.3253	0.0054	<.0001	<.0001
8						0.0368	0.1214	0.0820	0.1308	0.0129	-0.0654	-0.063	0.0065	0.0028	0.0673
						0.2964	0.0005	0.0198	0.0002	0.7146	0.0630	0.0718	0.8529	0.9362	0.0560
10							-0.097	0.6533	-0.090	-0.016	0.0224	-0.117	0.0099	-0.108	-0.171
							0.0057	<.0001	0.0100	0.6365	0.5250	0.0009	0.7785	0.0020	<.0001
11								0.0396	0.5768	0.1787	-0.038	0.0863	0.0868	0.3415	0.1446
								0.2607	<.0001	<.0001	0.2797	0.0142	0.0136	<.0001	<.0001
13									0.0327	0.0292	0.0194	-0.135	0.0656	-0.022	-0.068
									0.3531	0.4072	0.5817	0.0001	0.0625	0.5207	0.0535
14										0.1633	-0.043	0.1133	0.0568	0.3048	0.1964
										<.0001	0.2157	0.0013	0.1071	<.0001	<.0001
15											0.3263	0.1013	0.1656	0.1264	0.2149
											<.0001	0.0040	<.0001	0.0003	<.0001
16												0.0998	0.0594	0.0865	0.1228
												0.0045	0.0918	0.0139	0.0005
18													0.2399	0.0014	0.2038
													<.0001	0.9687	<.0001
19														-0.056	0.1496
														0.1082	<.0001
20															0.2024
															<.0001

Appendix 5. Spearman correlation (r_s) for relationships among morphological traits of spring wheat lines grown in the laboratory. The traits were measured at post-anthesis stage. See Table 4.1 for description of the traits. Correlations are significant at $P < 0.05$.

Trait No.	6	7	8	10	11	13	14	15	16	18	19	20	21
5	-0.0954	-0.0129	-0.0782	-0.1485	0.0573	-0.1022	0.0207	-0.0740	0.0361	0.1000	0.0478	0.0140	0.0832
	0.0112	0.7314	0.0377	<.0001	0.1286	0.0066	0.5833	0.0491	0.3377	0.0082	0.2067	0.7117	0.0275
6			0.0856	-0.1035	0.4570	-0.0228	0.4480	0.1439	-0.1089	0.0337	0.0680	0.3212	0.0972
			0.0229	0.0059	<.0001	0.5465	<.0001	0.0001	0.0037	0.3731	0.0720	<.0001	0.0100
7			0.1117	-0.1992	0.3037	-0.0525	0.3019	0.1999	-0.0519	0.0801	0.1389	0.2379	0.3442
			0.0029	<.0001	<.0001	0.1638	<.0001	<.0001	0.1677	0.0342	0.0002	<.0001	<.0001
8				-0.1087	0.0922	-0.0335	0.0639	-0.0140	-0.0116	-0.0439	0.0870	0.0504	0.1189
				0.0038	0.0143	0.3749	0.0894	0.7101	0.7573	0.2461	0.0213	0.1825	0.0016
10					-0.1032	0.6211	-0.1120	0.0542	0.0939	-0.1132	-0.0262	-0.1529	-0.1361
					0.0060	<.0001	0.0029	0.1501	0.0125	0.0027	0.4883	<.0001	0.0003
11						0.0638	0.5288	0.1605	-0.0502	-0.0374	0.0381	0.3287	0.1373
						0.0905	<.0001	<.0001	0.1825	0.3237	0.3145	<.0001	0.0003
13							0.0242	0.0574	0.0321	-0.0802	0.0084	0.0319	-0.0892
							0.5207	0.1277	0.3953	0.0342	0.8239	0.3988	0.0183
14								0.1467	-0.0533	0.0506	0.0930	0.3222	0.1670
								<.0001	0.1571	0.1818	0.0138	<.0001	<.0001
15									0.2614	-0.0245	0.0859	0.1825	0.1047
									<.0001	0.5181	0.0230	<.0001	0.0055
16										0.0508	0.0871	0.0242	0.0590
										0.1795	0.0211	0.5218	0.1181
18											0.2416	-0.0894	0.0926
											<.0001	0.0181	0.0143
19												0.0133	0.1365
												0.7261	0.0003
20													0.2052
													<.0001