INTERACTIONS AMONG BIOLOGICAL CONTROL, CULTURAL CONTROL AND BARLEY RESISTANCE TO THE RUSSIAN WHEAT APHID, *DIURAPHIS NOXIA* (KURDJUMOV), IN COLORADO, KANSAS AND NEBRASKA

by

PAOLA ANDREA SOTELO-CARDONA

B. Sc. Universidad del Valle, Colombia 2004
M.Sc. Universidad Nacional de Colombia, Sede Palmira 2010

AN ABSTRACT OF A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Entomology
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2010

Abstract

The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae) (RWA), is an important pest in the U.S. Western Plains, causing hundreds of millions of dollars of losses to wheat and barley production through reduced yields and insecticide application costs. The objectives of this research were to evaluate the performance of two RWA-resistant barley varieties planted approximately one month earlier than normal in experimental fields at Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska during 2007, 2008, and 2009. The experimental design was a split-plot design with two main plot treatments (early and normal planting dates), and four split plot treatments (barley varieties) that were randomized within each main treatment plot. The varieties included two RWA-barley resistant varieties, Sidney and Stoneham, and the susceptible variety, Otis, under thiamethoxam-protected and unprotected regimes. Sampling of RWA, other cereal aphids, and natural enemy populations was conducted on four dates from mid May through early July. RWA populations collected from early-planted plots (first week of March) were significantly lower than normal-planted plots in 2007-2009 at the Fort Collins, Colorado and Tribune, Kansas sites. In samples collected from early planting date plots, RWA-resistant varieties yielded RWA populations similar to those found on the insecticide-treated susceptible variety at both Fort Collins and Tribune. At the Sidney, Nebraska site, very low RWA populations were present and there were no differences between either planting date or varietal treatments. The combined effect of early planting and RWA-resistant varieties reduced RWA populations at the Fort Collins, Colorado site in all three years. Results were similar at the Tribune, Kansas site in 2007, but differences due to planting date or variety were not observed in 2008 or 2009. The lowest RWA populations occurred at the Sidney, Nebraska site, were independent of planting date and varietal treatments. The RWA-resistant barley varieties had no negative impact on populations of other cereal aphids compared to those found on the susceptible variety, Otis at any of the three research sites. The only treatment effective in reducing other cereal aphids was the insecticide, thiamethoxam. There was also no clear response of populations of other cereal aphids to different planting date. Neither the RWA-resistant barley varieties nor the systemic, short residual action insecticide treatment had adverse affects on the abundance of natural enemies.

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Dedication

I dedicate this research to the loving memory of my grandmother, Pola Sotelo, who was a very giving, caring and lovely woman. Her strength has been a great example during my life.

I also dedicate this research to my family, especially to my parents Guillermo, and Patricia; my brothers, Luis Felipe, and Rodrigo; and my lovely husband, Carlos. I am what I am, because of you.

"Try not to become a man of success, but rather try to become a man of value."-- Albert Einstein

CHAPTER 1 - BIBLIOGRAPHIC REVIEW OF THE RUSSIAN WHEAT APHID

Taxonomy and Distribution of Russian Wheat Aphid

The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (RWA), belongs to the order Hemiptera, family Aphididae (Table 1.1). RWA is native to the steppe country of southern Russia and the Mediterranean region and was first identified in Russia by Grossheim in 1900 (Grossheim 1914, Budak et al. 1999). This aphid has dispersed to other cereal-producing countries including Spain (1945), Turkey (1962), South Africa (1978), Mexico (1980), the USA (1986), Chile (1987), Canada (1990), Argentina (1992), and the Czech Republic (1995) (Webster et al. 1987, Damsteegt et al. 1992, Nowierski and Johnson 1995, Starý 1996, Reviriego et al. 2006) (Fig 1.1).

Table 1.1 Classification of *Diuraphis noxia* (Kurdjumov)

Order	Hemiptera
Suborder	Sterhnorrhyncha
Superfamily	Aphidoidea
Family	Aphididae
Subfamily	Aphidinae
Tribe	Macrosiphini
Genus	*Diuraphis*

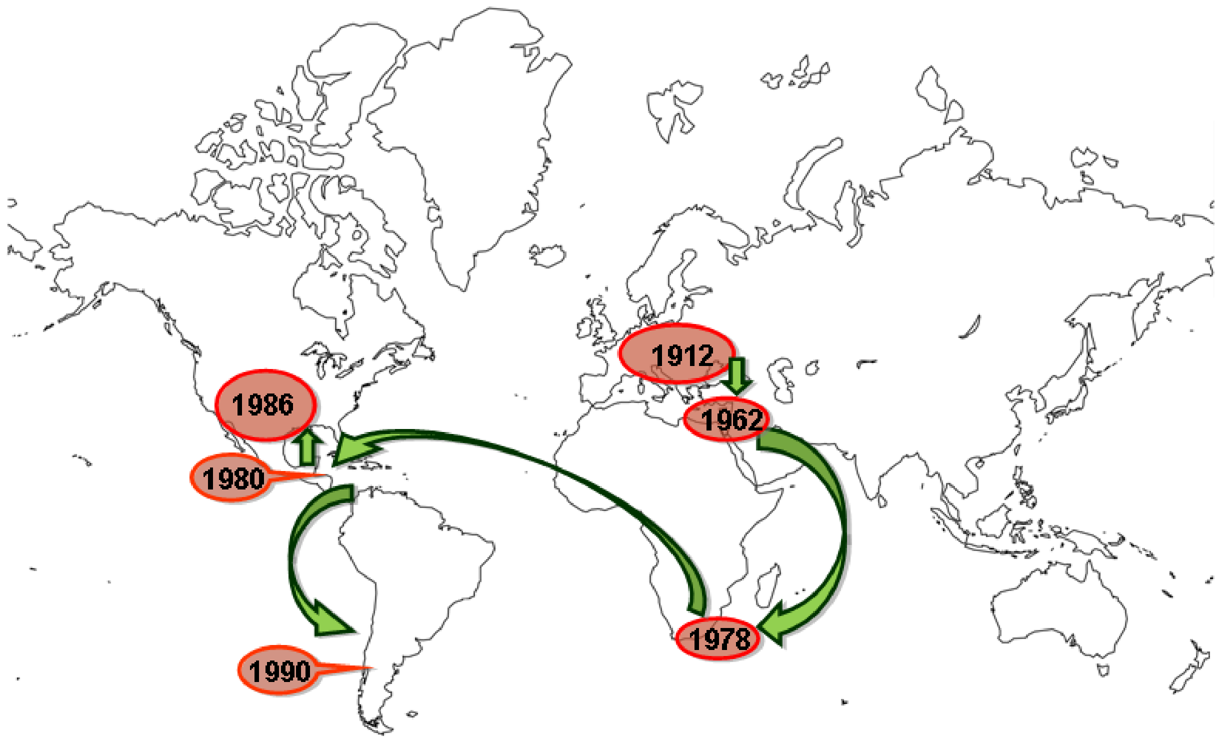

Fig. 1.1 Geographic distribution of the RWA. RWA has spread from southern Russia to countries with significant amounts of small grain production.
http://www.freeusandworldmaps.com

Since its initial introduction to the U.S., RWA has been recorded in more than 16 states (AZ, CA, CO, ID, KS, MT, ND, NE, NM, NV, OK, OR, SD, TX, UT, WA, WY), and three Canadian provinces (Alberta, Manitoba, and Saskatchewan) (Reed et al. 1992; Brewer and Elliot 2004). The distribution of RWA in the U.S. corresponds to the geographic area known as the Great Plains that includes the 16 western U.S. states and the Canadian provinces of Alberta, Saskatchewan and Manitoba (Fig. 1.2). The High Plains are elevated regions in the Great Plains characterized as a semi-arid steppe, with grassland-predominant vegetation and with extended periods of drought. In these regions, heavy RWA infestations of barley and wheat crops are associated with dry areas (300-400 mm summer rainfall) (Hughes 1988).

Fig. 1.2 Distribution of the RWA in the U.S. Climatic and geologic conditions in the Great Plains are suitable for the development and spread of the RWA.

Map of the Great Plains. Used by permission of the Center for Great Plains Studies at the University of Nebraska-Lincoln; website: **http://www.unl.edu/plains/about/map.shtml**

Identification of Russian Wheat Aphid and Damage Symptoms

In 1987, Stoetzel identified the RWA as a yellow-green aphid, small (<2.3 mm), with inconspicuous antennal and body hairs, short cornicles (as long as wide), elongate cauda and supracaudal processes present on the dorsal side of the eight abdominal tergite (Fig. 1.3a). Based on the use of host plants, RWA can be identified as a non-host alternating aphid or monoecious, meaning that RWA can remain on closely related species of Poaceae throughout the year (anholocyclic).

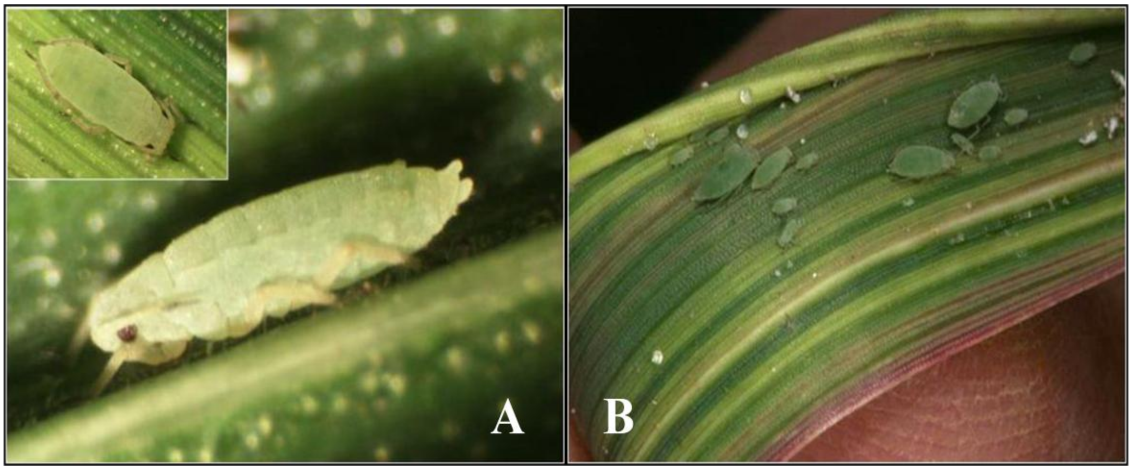

Fig. 1.3 Russian wheat aphid and damage caused to small grain plant leaves. a) Adult aphid and b) Longitudinal white leaf streaking.

Note the short cornicles and the “double-tail” (elongate cauda and supracaudal processes). Taken and adapted from: www.entomology.ksu.edu

Pictures courtesy of the author: Phillip Sloderbeck, Area Extension Director – Southwest Kansas State University Southwest Research and Extension Center

The RWA populations are composed of females that reproduce asexually and give birth to live daughters (thelytoky). Eggs are unnecessary for RWA overwintering. This condition has been observed for populations in South Africa, Canada, and in the United States (Mezey and Szalay-Marzsó 2001, Williams and Dixon 2007). Sexually reproducing populations can be found in Europe and central-western Asia, with overwintering egg stage, fundatrices emerging early in the spring, and sexual forms appearing in about mid-autumn (Starý 1999). Based on the availability of resources, RWA can be alate or wingless. This insect has four or five molts depending on temperature, with an ideal range of 16-21^{o}C for the development of the nymphal stages through the reproductive adult stage (Hein et al. 1998, Michaud and Sloderbeck 2005).

RWA fecundity varies from 32 to 82 nymphs per female at 13°C and 17°C, respectively (Aalbersberg et al. 1987, Nowierski and Johnson 1995). Hughes (1988) commented that development and reproductive rates are linearly related to temperature between 2°C and 25°C, and below or above those limits reproduction and development are null.

Quisenberry and Ni (2007) classified the common injury symptoms produced on crops by Aphidoidea, and suggested that injuries caused by RWA can be either desistance or deformation. Desistance (ceasing growth) can be the result of aphid-induced chlorosis and stunting (Burd et al. 1993), and deformation includes misshapen fruits (Peairs 1998) and pseudogalling (Burd et al. 1993). The RWA is a phloem feeder that injects salivary enzymes into the host to produce characteristic damage consisting of leaf rolling in young leaves, prevention of unrolling of developing leaves, purple discoloration, prostrate growth, longitudinal white leaf streaking, deformed grain spikes, reduction in the photosynthetic capacity by destruction of chloroplasts and a subsequent reduction in grain yield (Riedell 1989, Smith et al. 1991, Estakhr and Assad 2002, Heng-Moss et al. 2003, Mornhinweg et al. 2006, Saheed et al. 2007) (Fig. 1.3b). Saheed et al. (2010) suggested that the symptoms shown in RWA-infested plants are due to the ability of the aphid in inflict severe damage on the phloem transport system, with disruption and diversion of assimilates in barley leaves that appears to be responsible for yield losses ranging from 30 to 60%. The reduction in the transport of assimilates starts as early as 72 h after RWA infestation and prolonged feeding may result in total cessation of phloem transport. RWA caused economic losses of more than $1 billion to U.S. agriculture by the late 1990s in wheat and barley production through reduced yields and pesticide treatment cost (Webster et al. 2000, Michaud and Sloderbeck 2005).

At the beginning of the growing season, RWA population growth rate is slow, but increases during tillering and stem elongation, with population growth occurring from the boot stage on. Significant yield reduction occurs when biotic and abiotic conditions are optimal for rapid population development, growth and outbreaks (Hughes 1988, Nowierski and Johnson 1995, Backoulou et al. 2010). Field records from South Africa and the United States suggest that areas with high rainfall are not favorable for infestation by RWA. Populations appear to persist best in warm regions with low soil moisture (up to 60 mm) and low rainfall (Hughes 1988, Hughes and Maywald 1990).

Russian wheat aphid populations virulent to different wheat plant resistance genes have been documented in Hungary, Russia, South Africa, and the U.S. (Puterka et al. 2007). A biotype, as defined by Weiland et al. (2008) is a population of RWA that is able to feed successfully on a host plant genotype previously resistant to such infestation. After the initial introduction of RWA to the U.S., only one RWA biotype (RWA1) was known. A second biotype (RWA2) was reported in Colorado in 2003 with virulence to all known genes in resistant wheat sources (*Dn1, Dn2, Dn4, Dn5, Dn6, Dn8, Dn9, Dnx* and *Dny*) with the exception of genotypes containing the *Dn7* gene (Marais et al. 1994, Haley et al. 2004, Burd et al. 2006, Puterka et al. 2006, Lapitan et al. 2007a, Puterka et al., 2007). Since 2003, at least eight biotypes of RWA have been identified in the U.S. (Burd et al. 2006, Lapitan et al. 2007b, Weiland et al. 2008, Zaayman et al. 2009). Results of Zaayman et al. (2009) suggest that each RWA biotype has different eliciting agents (soluble proteins as suggested by Lapitan et al. 2007b) that interact in a highly specific manner with plant resistant genes and lead to the activation of specific defense pathways for that particular interaction.

A survey of the distribution and diversity of U.S. biotypes by Puterka et al. (2007) indicated that RWA1 and RWA2 constituted 27.2% and 72.8% of all samples respectively, collected in Oklahoma, Texas, New Mexico, Colorado, Kansas, Nebraska, and Wyoming. Randolph et al. (2009) studied virulence in seven RWA biotypes, showing that RWA1 is the least virulent (Weiland et al. 2008), RWA4, 5, 6 and 7 possess intermediate levels of virulence, RWA3 is highly virulent, and that RWA2 is the most virulent biotype with susceptible responses to 12 plant differentials and intermediate response to five plant differentials.

Host Plants of Russian Wheat Aphid

Infestations of RWA are heavy in monocultures of wheat *(Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and triticale (*Triticosecale rimpaui* Wittm) crops, but at least 40 species of small grains and cool-season grasses, including oats (*Avena sativa* L.), Spanish brome (*Bromus madritensis* L.), tall wheatgrass (*Elytrigia elongate* (Host) Nevski), rice (*Oryza sativa* L.), canarygrass (*Phalaris canariensis* L.), and rye (*Secale cereale* L.) are considered suitable RWA hosts (Stoetzel 1987, Hughes 1988, Hughes and Maywald 1990, Brewer and Elliot 2004, Weiland et al. 2009, Zaayman et al. 2009). Rye and wheats (*Triticum* spp.) are the cultivated cereals most related to barley and are grouped in the tribe Triticeae (Hordeae). In 1997, *P. canariensis* was detected as one of the most preferred hosts of *D. noxia* in the Konya province of Turkey (Elmali 1998). This plant remains green throughout the summer with *D. noxia* reaching high populations, beginning in the tillering stage and peaking in the heading stage (Uysal and Turanli 2004). This observation is important for U.S. barley production because *P. canariensis* is an annual grass that is highly distributed in 41 states and eventually could be used as an alternate host by *D. noxia* during the summer.

Recent studies (Haley et al. 2004, Weiland et al. 2008, Weiland et al. 2009) have confirmed the presence of some biotypes on non-cultivated grasses in the interval between summer harvest and fall planting. In Colorado, crested wheatgrass (*Agropyron cristatum* (L.) Gaerth), downy brome (*Bromus tectorum* L.), Canada wildrye (*Elymus Canadensis* L.), and intermediate wheatgrass remain the most consistent RWA alternate hosts identified by Weiland et al. (2009).

Summarizing, the main reasons for the success of RWA and its rapid spread in the western United States are the presence of suitable hosts, the adaptation of the aphid to the arid conditions in the High Plains, the development of biotypes that are virulent to previous sources of resistance, the aphid's asexual mode of reproduction, a capacity to overwinter as an adult, and the protection found in the aphid-induced rolled leaf (pseudo-gall) which creates a tubular refuge that protects the aphid from chemical treatment and natural enemies.

Barley: A Preferred Host of Russian Wheat Aphid

Barley, *Hordeum vulgare* L., belongs to the tribe Triticiae, with all the species in the genus *Hordeum* presenting similar morphological and diagnostic characters. However differences in the chromosome numbers and life forms (annual vs. perennial) are also apparent within the genus. Cultivated barley (*H. vulgare* and other 20 species) is diploid (2n=2x=14), but tetraploids (2n=2x=28) (*H. jubatum* L., *H. manrinum* Huds., *H. secalinum* Schreb., *H. capense* Thunb., etc.) and hexaploids (2n=6x=42) (*H. brevisubulatum* (Trin.) Link, *H. brachyantherun* Nevski, *H. parodii* Covas, etc.) also exist (von Bothmer et al. 2003).

Archaeological evidence suggests that domestication of barley took place around 10,000 B. P. in the Fertile Crescent of the Middle East region. In an extensive review, Molina-Cano et al. (2002) discussed the “new view on the origin of cultivated barley” which put in context all historical knowledge about centers of origin and phylogenetic studies based on RFLP techniques. These results have led to a new multicentric barley origin hypothesis which includes domestication from Morocco through Tibet, including the Near East, Ethiopia, and the western Mediterranean region (Molina-Cano et al. 2002).

Barley is an important economic crop and is the fourth largest grain crop (production area) in the U.S. behind wheat, rice, and corn. Barley is an annual grass that can be grown in winter and spring. Winter plants need vernalization in order to produce flowers and set seed but spring plants do not. Spring barley matures early, uses less water than wheat, can easily be rotated with winter wheat, and is adapted to dry and cold climates (Magness et al. 1971).

From 1994 to 2003, U.S. barley production was valued at approximately $760 million for animal food (51%) and malting (44%). Only 3% and 2 % of U.S. produced barley is dedicated to production of seed and human food, respectively (National Barley Growers Association). However, one of the main limitations for barley production in the U.S. is the presence of RWA in western states. Before the introduction of RWA, barley production was approximately 4.5 million of hectares, but after the introduction of RWA, production decreased dramatically to only 1.2 million hectares in 2006 (FAO 2008) (Fig. 1.4). A similar trend is observed in particular for the Western Plains (Fig. 1.5). Until the introduction of RWA, feed barley was used as the alternate crop in winter wheat rotations and valued as an irrigated feed crop (Mornhinweg et al. 2009). However, all barley cultivars used in commercial production were highly susceptible to RWA feeding damage, including Otis (PI 8775), a feed barley cultivar released in 1951 with high yields under dryland conditions (Mornhinweg et al. 2002, Mornhinweg et al. 2009). The dramatic reductions in U.S. barley production due to RWA strongly suggest the need for the development and implementation of a barley Russian wheat aphid Integrated Pest Management (IPM) strategy to restore barley production and fulfill producer requirements in environmentally clean conditions.

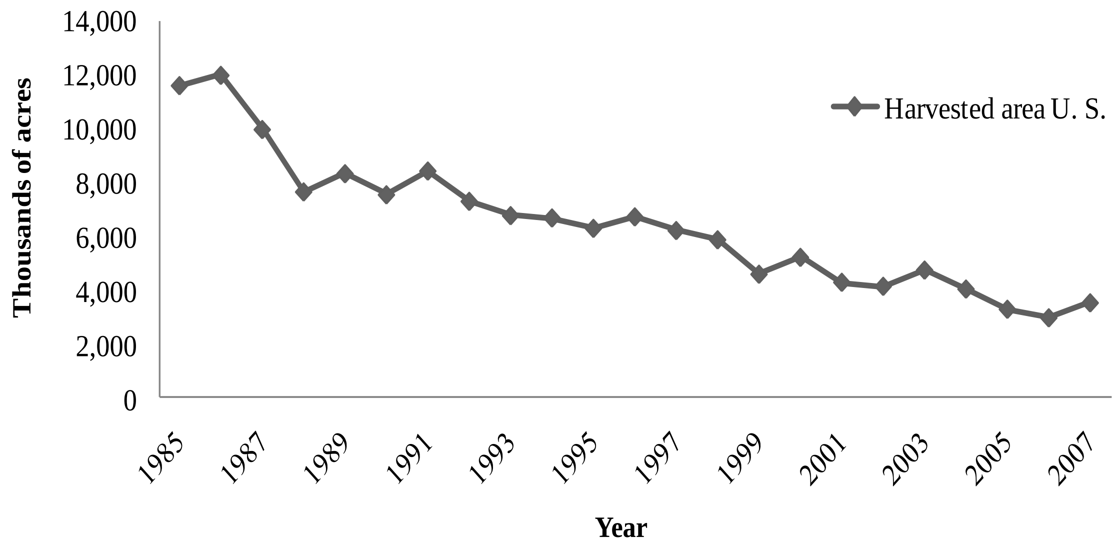

Fig. 1.4 Harvested area of barley in the United States.

Source FAOSTAT | © FAO Statistics Division, 2008.

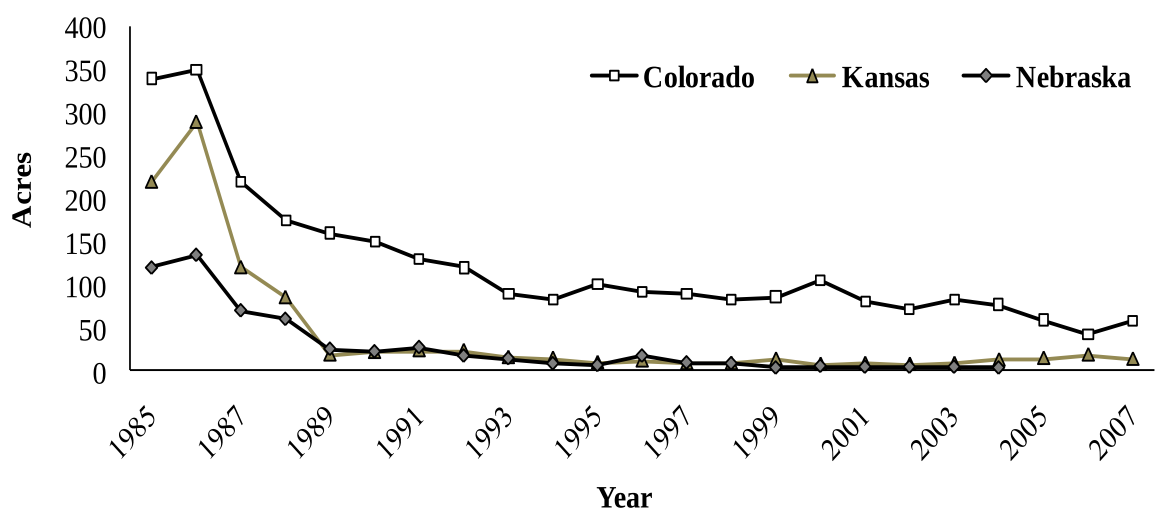

Fig. 1.5 Harvested area of barley in the Western Plains.

Source FAOSTAT | © FAO Statistics Division, 2008.

Integrated Management of Russian Wheat Aphid

The interest of barley producers is to decrease losses with the use of varieties with RWA-resistance or by reducing the high costs associated with insecticide application. The needs of barley producers can be addressed with the use of an IPM strategy consisting of the rational use of chemical control, biological control, cultural control and host plant resistance (Miller and Pike 2002, Zaayman et al. 2009). As stated by Holtzer et al. (1996), IPM for RWA based on the use of biological control, cultural practices and host plant resistance is important because of their compatibility with producer goals of water and soil conservation in the Great Plains.

Chemical Control

The modern era of chemical control of insect pests began on 1939 with discovery of insecticidal properties of a compound known as dichloro-diphenyl-trichloroethane (DDT) by Paul Muller, who was awarded a Nobel Prize in 1948 (Dhaliwal et al. 2004). The main features of insecticides at that time were high efficiency, which lead to high levels of pest population reduction and corresponding increases in crop production. However, the improper use of insecticides caused serious adverse effects on human health and the environment. Dhaliwal et al. (2004) stated that approximately 25 million agricultural workers are poisoned every year by the use of insecticides. Currently, the use of chemical control is determined by economic thresholds, which avoid the use of broad-spectrum insecticides, and promotes the action of biological agents as natural enemies in the control of insect pests (Stern et al. 1959, van Emden 2002).

Chemical control has been extensively used to kill and reduce the populations of insect pests of many economically important crops worldwide. Insecticides used for the control of aphids can be categorized by their mode of action. Organophosphates and carbamates that act as acetylcholinesterase (AchE) inhibitors are highly toxic to mammals (Plapp 1991, Dewar 2007). Pyrethroids act on the voltage-gated sodium channel, have low mammalian toxicity, but are highly toxic to fish (Dewar 2007). Neonicotinoids act on the post-synaptic nicotinic acetylcholine-receptors (nAChR), and cause aphids paralysis and death. Neonicotinoids have low mammalian toxicity but are highly toxic to birds (Cox 2001, Dewar 2007). Pymetrozines are highly specific to aphids because they affect the nerves that control the salivary pump of sucking insects and eventually cause death by starvation (Schwinger et al. 1994, Dewar 2007). Other aphicides are diafenthiuron and triazamate, which inhibit ATPases (respiration) and acetylcholinesterases, respectively (Dewar 2007). Wheat, barley and other cereals are protected with systemic insecticides like neonicotinoids because they offer good protection against RWA and other cereal aphids when applied as seed treatments (Miller and Pike 2002). Chemical control was the primary management strategy for RWA until RWA-resistant cultivars were developed, specifically the release in 1994 of 'Halt' which contains the *Dn4* resistance gene (Quick et al. 1996, Puterka et al. 2007, Shufran and Payton 2009).

Biological Control

Biological control is part of a broader category known as natural control, where populations are regulated within lower and upper limits by abiotic factors such as weather and spatial requirements, or biotic factors such as natural enemies and intra- and interspecific competition. Biological control of pest species by natural enemies operates in a density dependent manner. If the density of a pest population increases, the intensity of biological control increases as well, but if pest population density decreases, the intensity of biological control decreases correspondingly. Thus, outbreaks of pest species can be kept in check if pest populations remain at levels sufficient to sustain natural enemies (Debach and Rosen 1991).

Biological control is defined as the use of living organisms such insects, virus, bacteria, fungi, vertebrates, and plants to suppress the population of a specific pest organism, making it less abundant or less damaging than it would otherwise be (Eilenberg et al. 2001). Pedigo and Rice (2006) consider biological control to be one of the oldest and most effective means of insect control. The objective of biological control is to manipulate natural enemies (introduced or native) in order to regulate pest populations to a non-economically important level, or to a level of low economic impact (Pedigo and Rice 2006).

The general attributes characterizing successful biological control agents are host specificity, synchrony with the pest, a high intrinsic rate of increase, an ability to survive periods with few or no prey, and good searching ability (Hajek 2004). However, it is important to note some disadvantages of biological control, which include failure to control the host population before host economic damage occurs, poor adaptation to the environment of the host, and susceptibility to insecticidal and cultural control (Hajek 2004).

Interest in biological control increased greatly in the late 1980's after a dramatic reduction in U.S. wheat production due to RWA. Wraight et al. (1993) found that parasite prevalence was lower than 5% in Colorado, and the most common parasitic wasp was *Diaeretiella rapae* (M'Intosh). They also suggested that other native biological control agents were ineffective in reducing RWA populations, and that the only way to control aphid populations was by initiation of a classical biological approach against RWA (Wraight et al. 1993, Lee et al. 2005). Several hundred species of predators and parasitoids were released in 18 U.S. states and Canada from 1987 to 1997 after the USDA 'National RWA Integrated Pest Management Program' was initiated (Brewer and Elliot 2004, Lee et al. 2005, Powell and Pell 2007). As stated by Michaud and Sloderbeck (2005), this biological control effort involved more than 120 scientists from 20 countries, who imported beneficial insects of at least 24 species in 16 affected states in the United States. Currently, some of the most important biological control agents are braconid parasitoids (*Aphidius*, *Lysiphlebus*, and *Aphelinus*) (Hymenoptera: Braconidae), ladybird beetles (*Adalia*, *Coleomegilla*, *Harmonia*, *Hippodamia*) (Coleoptera: Coccinellidae), lacewing (Neuroptera: Chrysopidae, Hemerobiidae), predatory bugs (Hemiptera: Anthocoridae, Geocoridae, and Nabidae). Various entomopathogenic fungi including *Beauveria bassiana*, *Paecilomyces fumosoroseus*, and *Lecanicillium* spp., can infect RWA, but most require substantial humidity to be effective, which makes them a less likely to cause mortality in arid regions where the aphid is most prevalent (Elliot et al. 1998, Michels et al. 2001, Powell and Pell 2007).

Parasitoids introduced from Eurasia and Morocco included *Aphelinus albipodus* Hayat & Fatima, *Aphelinus asychis* Walker, *Aphelinus varipes* (Foerster) (Hymenoptera: Aphelinidae), *Aphidius colemani* (Viereck), *Aphidius matricariae* Haliday, *Aphidius picipes* (Nees), *D. rapae* and *Ephedrus plagiator* (Ness) (Hymenoptera: Braconidae). In north central Colorado, southeastern Wyoming, and southwestern Nebraska, at least ten parasitoids were released, but only *A. albipodus*, and *D. rapae* were abundant by early to mid 1990s. (Brewer and Elliot 2004).

Predators have been extensively used since 1900 for the control of several aphid species, as well as whiteflies, mealybugs, scales and mites. For that reason, currently more than 179 coccinellid species have been introduced to the U.S. (Obrycki and Kring 1998, Powell and Pell

2007). Also in the particular case of predators for RWA, Miller (1995) demonstrated that a South American coccinellid, *Eriopis connexa* Mulsant, was a viable predator of RWA with optimal survival at 34°C in addition to the predators introduced from the native area of this insect pest.

Bosque-Perez et al. (2002) monitored the species composition, relative abundance, and seasonal dynamics of aphid natural enemies on spring wheat that was resistant and susceptible to RWA in Moscow, Idaho in 1997 and 1998. The coccinelids *Hippodamia convergens* Guèrin-Mèneville, *Coccinella septempunctata* L., *C. transversoguttata* Brown and *C. trifasciata* Mulsant were detected. However, no significant differences in adult or immature coccinellid densities were observed between the resistant and susceptible genotypes. The most abundant primary hymenopteran parasitoids were *D. rapae*, *Aphidius ervi* Haliday, *A. avenaphis* (Fitch), and *Lysiphlebus testaceipes* (Cresson).

Lee et al. (2005) evaluated the effectiveness of predators and parasitoids of RWA using mechanical exclusion in winter wheat fields in southeastern Colorado. *Hippodamia convergens*, and the generalist *Nabis* spp. (Hemiptera) were the most abundant predators, but did not substantially reduce RWA numbers. Similarly, *H. convergens*, *C. septempuntata* L., and *H. sinuata* Mulsant were the most abundant predators during declining RWA population growth. The dominant parasitoid was *L. testaceipes*, but parasitism rates were very low.

Michaud and Sloderbeck (2005) reported that *H. convergens*, also the key predator of greenbug, *Schizaphis graminum* Rondani, is one of the most important RWA natural control agents in western Kansas. *Coccinella septempunctata* is common in wheat fields in early spring and may play a role in reducing RWA numbers. Similarly, aphidiid wasps, including the greenbug parasitoid *L. testaceipes,* also attack and develop in RWA. Small lady beetle species in the genus *Scymnus* have larvae with distinctive waxy filaments and can also be found feeding in RWA colonies. The introduced parasitic wasp *Aphelinus albipodus* (Hymenoptera Aphelinidae), and other native *Aphelinus* spp. are small, and can forage in an efficient way rolled leaves inhabited by RWA.

Cultural Control

Cultural control involves the manipulation of the environment to create a less favorable habitat for the pest population (Elzinga 2000), which disrupts the life cycle of the pest or improves conditions for natural enemies (van Emden 2002). Although resistant varieties and insecticides provide the most effective RWA control, several other practices can help minimize the need for chemical applications. Cultural practices can be grouped in three categories: prevention, avoidance and suppression. The prevention and avoidance categories include the use of pest-free seed; the selection of well-adapted cultivars; optimal plant nutrition, water management, and sanitation; crop rotation; selection of "pest-free" planting and harvesting times and trap crops (Bajwa and Cogan 2004). For suppression, the main practices are crop diversification, soil tillage, destruction of alternative hosts and volunteer-crop plants, and optimal row spacing (Bajwa and Cogan 2004). For RWA management, the control of volunteer wheat and barley is essential, as these are the most important source of infestation for the new fall crop. A 3-week volunteer-free period is necessary prior to emergence of fall seedlings. Adjusting planting dates by planting winter wheat as late as possible and planting spring grain as early as possible also help manage RWA. Since RWA often gets its start in stressed portions of fields, proper soil fertilization is necessary for the production of a healthy and stress-free crop. Finally, the use of treated seed with insecticide and the selection of a variety that is well-adapted to local growing conditions also help avoid RWA infestation (Peairs 1998).

Host Plant Resistance

One of the recent definitions of plant resistance to insects given by Smith (2005) is "Plant resistance to arthropods is the sum of the constitutive, genetically inherited qualities that result in a plant of one cultivar or species being less damaged than a susceptible plant lacking these qualities". The 'inherited qualities' present in resistant plants can be grouped in three categories: antixenosis, antibiosis, and tolerance.

Antixenosis is described as non-preference for feeding or oviposition in resistant plants that can be the result of morphological or chemical factors in the plant creating repellence to insects (Smith 2005). It is important to note that this category of resistance affects the behavior but not the physiology of the insect. The second category, antibiosis resistance, is described as the deleterious effects on an insect pest's life cycle, growth, development, or reproduction as a consequence of feeding on resistant plants (Smith 2005). The third category is tolerance, defined as the ability of the infested plant to recover from damage caused by a population of an insect pest when compared with a susceptible plant (Smith 2005).

Genetics of Barley Plant Resistance to Russian Wheat Aphid

From 1990 to 1993, 24,000 barley accessions were evaluated for resistance by USDA-ARS researchers at Stillwater, OK. Of these, 109 accessions presented some level of resistance and the main category of resistance expressed was tolerance (Mornhinweg et al. 2006). Two of these accessions were advanced to produce the RWA-resistant lines STARS-9301B and STARS-9577B (Mornhinweg et al. 1995, 1999). Resistance in STARS 9301B is conferred by one incompletely dominant gene and one dominant gene with epistasis (Mornhinweg et al. 1995). This source of resistance was used as the resistant parent in crosses that resulted in the release of the cultivars ‘Burton’ and ‘Sidney’ (Bregitzer et al. 2005). Resistance in STARS 9577B is conferred by two dominant genes with recessive epistasis (Mornhinweg et al. 1999, Bregitzer et al. 2008). Two new releases, Sidney and Stoneham, were obtained from crosses between ‘Otis’, a commercial but highly RWA susceptible variety, and STARS 9301B and STARS 9577B, respectively (Bregitzer et al. 2008, Mittal et al. 2009). Most important, the resistance in STARS 9301B and STARS 9577B is effective against five *D. noxia* North American biotypes (Puterka et al. 2006, Weiland et al. 2008). However, STARS-9301B has a greater level of resistance than STARS-9577B (Mornhwinweg et al. 2006, Mittal et al. 2009). STARS-9577B resistance is linked to two quantitative trait loci (QTL), one located on the short arm of chromosome 1H and the other on the long arm of chromosome 3H (Mittal et al. 2009). STARS-9301B resistance is linked to the two QTLs associated with STARS-9577B resistance as well as a QTL on chromosome 2H (Nieto-Lopez and Blake 1994, Mittal et al. 2008). The spring barley cultivar RWA 1758, obtained from the cross ‘Baronesse’*4/STARS 9577B, also contains RWA resistance (Bregitzer et al. 2008).

Research Justification

Two golden rules were suggested by van Emden (2002) for Integrated Pest Management as follows:

1. *If a single method gives adequate control on its own, then there is a danger of a tolerant pest strain increasing in gene frequency and no opportunity to use a second method in addition. The method therefore needs to be made less efficient (reduced dose of pesticide, partial host-plant resistance rather than immunity) for there to be value in introducing another control method to supplement it.*

2. *Methods are increasingly worth combining to the extent that the control then achieved exceeds the additive effects of the two methods in isolation.*

In addition, van Emden (2007) described the possible interactions of two or three aphid IPM strategies, and cited examples of interactions between chemical and biological control (Acheampong and Stark 2004) and between biological control and host-plant resistance (Sotherton and Lee 1988). A three-way interaction between chemical control (malathion), host plant resistance ('Rapier' wheat) and biological control (parasitoids and coccinellids) for the management of the rose-grain aphid, *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae) has also been developed (Tilahun and van Emden, 1997; van Emden 2007). In this system, natural enemies are more tolerant to chemical insecticides when reared in aphids feeding on a resistant variety than in aphids feeding on the susceptible wheat variety 'Maris Huntsman'. Results from studies of the compatibility of plant resistance to RWA and biological control are variable. Reed et al. (1991, 1992) found a negative interaction between biological control and plant resistance to RWA caused by high levels of antibiosis that affected populations of both RWA and the parasitoid *D. rapae*. However, results of Farid et al. (1997) and Brewer et al. (1998) suggest compatibility between plant resistance to RWA and two different natural enemies. Brewer et al. 1998 studied the effect of barley resistance on the RWA parasitoid's abundance. The authors found that abundance was similar in susceptible and resistant barley lines, concluding that the two management tactics were compatible and therefore can be used in combination in order to control RWA populations. These 'golden rules' and the previous examples of positive interactions among different IPM strategies support the proposed research because there is an increasing need to optimize current RWA management tactics, thereby improving barley production in the western High Plains and diminishing the costs associated with expensive chemical insecticide applications.

Objectives

1. To evaluate the effectiveness of an early planting date in the first week in March on the reduction of RWA populations compared to the current normal planting date in the first week in April.

2. To evaluate the effect of RWA-resistant barley varieties, Sidney and Stoneham, on reducing RWA populations when compared to a susceptible barley variety.

3. To evaluate the compatibility of an early planting date with the RWA-resistant barley varieties Sidney and Stoneham in reducing RWA populations.

4. To determine how other cereal aphids infesting barley are affected by RWA integrated pest management strategies.

5. To evaluate how changing to an early barley planting date and using RWA-resistant barley varieties Sidney and Stoneham affects the occurrence and of natural enemies.

Null hypotheses

1. There are no differences between RWA populations produced on crops planted at early- and normal planting dates.

2. There are no differences between RWA populations produced on susceptible and RWA-resistant barley varieties.

3. There are no differences in RWA populations as a result of the interactions between planting dates and barley varieties.

4. Populations of cereal grain aphids other than RWA are not affected by altered planting dates, RWA-resistant barley varieties, or planting date-variety interactions.

5. There is no difference in the natural occurrence of RWA biological control agents in early- and normal- planted plots of either RWA-resistant or susceptible barley varieties.

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CHAPTER 2 - EFFECTS OF EARLY BARLEY PLANTING AND RUSSIAN WHEAT APHID RESISTANT BARLEY VARIETIES ON RUSSIAN WHEAT APHID POPULATIONS IN COLORADO, KANSAS, AND NEBRASKA

Abstract

The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae) (RWA), is an important pest in the U.S. Western Plains, causing hundreds of millions of dollars of losses to wheat and barley production through reduced yields and insecticide application costs. The objectives of this research were to evaluate the performance of two RWA-resistant barley varieties planted approximately one month earlier than normal in experimental fields at Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska during 2007, 2008, and 2009. The experimental design was a split-plot design with two main plot treatments (early and normal planting dates), and four split plot treatments (barley varieties) that were randomized within each main treatment plot. The varieties included two RWA-barley resistant varieties, Sidney and Stoneham, and the susceptible variety, Otis, under thiamethoxam-protected and unprotected regimes. Sampling of RWA populations was conducted on four dates from late May through early July. Climatic conditions at Fort Collins, Tribune, and Sidney were very different and are likely responsible for differences in RWA populations at each location. High temperatures and medium levels of precipitation were conditions that appeared to create conditions most favorable to RWA outbreaks at Tribune. Early planted plots grew and developed faster than those planted one month later, and avoided high levels of RWA colonization. Early planting helped reduce RWA infestations at Fort Collins and Tribune in 2007. Further research is necessary to evaluate how drought stress affects the performance of both plants and aphids. The resistant varieties Sidney and Stoneham exhibited low to intermediate levels of RWA infestation that may be a product of tolerance and/or antibiosis resistance. RWA populations observed on Sidney and Stoneham were similar to those on Otis plants protected by seed treatment, suggesting that plant resistance is important in reducing RWA populations in the field. An additive combined effect

of planting date and variety was detected in the three years of research, and early-planted plots of Sidney and Stoneham sustained significantly lower RWA infestation levels when compared with Otis. Results of this research indicate that earlier than normal planting dates and RWA-resistant barley varieties can contribute significantly to reduced RWA infestations in eastern Colorado, western Kansas, and western Nebraska.

Key words: tolerance, early planting date, varietal effect, synergist effect, barley aphid

Introduction

The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), (Hemiptera: Aphididae) (RWA) is native to the steppe country of southern Russia and the Mediterranean region and was first identified in Russia by Grossheim in 1900 (Grossheim 1914, Budak et al. 1999). This aphid has dispersed to other cereal-producing countries including Spain (1945), Turkey (1962), South Africa (1978), Mexico (1980), the USA (1986), Chile (1987), Canada (1990), Argentina (1992), and the Czech Republic (1995) (Webster et al. 1987, Damsteegt et al. 1992, Nowierski and Johnson 1995, Starý 1996, Reviriego et al. 2006). Since its initial accidental introduction, RWA has become a limiting factor in barley and wheat production in the U.S. Western Plains, causing hundreds of millions of dollars of losses through reduced yields and increased insecticide treatment costs (Webster et al. 2000, Michaud and Sloderbeck 2005).

The RWA inflicts direct and indirect damage to the host plants. Direct damage consists of the dehydration of the plant by removal of phloem sap. Once phloem transport is affected, a disruption and diversion of assimilates is observed, causing yield losses as high as 60% (Saheed et al. 2010). Indirect damage inflicted by RWA consists of the injection of toxic saliva into the host, producing characteristic symptoms such as leaf rolling in young leaves, prevention of unrolling of developing leaves, purple discoloration, prostrate growth, longitudinal white leaf streaking, deformed spikes, and reduction of photosynthetic capacity by destruction of chloroplasts and a subsequent reduction in grain yield (Smith et al. 1991, Mornhinweg et al. 2006, Saheed et al. 2007).

Insecticidal control has been extensively used to kill insect pests of many economically important crops worldwide, including aphids (Dewar 2007). However, the misuse of insecticides has caused serious adverse effects on human health, with millions of agricultural workers being poisoned, and has also had detrimental effects on the environment (Dhaliwal et al. 2004). On the other hand, the use of IPM strategies such as biological control, cultural control and host plant resistance in combination with the rational use of insecticide applications is compatible with the goals of water and soil conservation in the Great Plains (Holtzer et al. 1996, Zaayman et al. 2009). Such an integrated management system appears to be a better approach for barley and wheat producers in order to reduce insecticide treatment cost and increase crop yields.

Examples of cultural control include the selection of well-adapted cultivars, planting and harvesting times, and trap crops (Bajwa and Cogan 2004). Peairs (1998) suggested that improved management of RWA infestations was possible by planting spring grain crops earlier than normal, the use of insecticide treated seed and finally, selection of varieties well-adapted to local growing conditions.

The RWA-resistant barley lines STARS 9301B and STARS 9577B have been used to develop RWA resistant cultivars, since the mid 1990s in the U.S. (Mornhinweg et al 1995, 1999). Resistance in STARS 9301B, conferred by one incompletely dominant and one dominant gene with epistasis, was used to develop the cultivars 'Burton' and 'Sidney' (Bregitzer et al. 2005, Mornhinweg et al. 2009). Resistance in STARS 9577B, conferred by two dominant genes with recessive epistasis, was used as the resistant parent in crosses to develop 'Stoneham' and 'RWA1158' (Bregitzer et al. 2008, Mittal et al. 2009). Two newly released varieties, Sidney and Stoneham were obtained from the cross of STARS 9301B and STARS 9577B respectively, with 'Otis' barley. Otis has been a good feed barley variety since its original introduction in 1951, because of its high yields under dryland conditions, but is highly susceptible to RWA infestations (Mornhinweg et al. 2002, Mornhinweg et al. 2009). STARS 9301B and STARS 9577B have high grain yields and the resistance genes in these breeding lines are effective against five RWA North American biotypes (Puterka et al. 2006, Weiland et al. 2008).

In order to determine the compatibility between early planting date, the use of RWA resistant barley varieties, and the performance of resistant varieties when compared with the use of rational insecticide applications, the objectives of this research were: 1) to evaluate RWA populations on an early planting date (first week in March) and a normal planting date (first week in April); 2) to evaluate differences between RWA populations produced on the susceptible barley variety Otis versus two RWA-resistant barley varieties; and 3) to evaluate the compatibility between the early planting date regime with the two RWA-resistant barley varieties.

Materials and Methods

Location

The present study was conducted at three locations within the Great Plains biogeographic region during 2007, 2008, and 2009. These study sites were located at the University of Nebraska High Plains Agricultural Laboratory near Sidney, Nebraska (41^{0}12’21.91’’ N, 103^{0}00’42.72” W, elevation: 1320 m), the Colorado State University Agricultural Research, Development and Education Center (ARDEC), near Fort Collins, Colorado (40^{0}39’09.37” N, 105^{0}00’01.67” W, elevation: 1550 m), and the Kansas State University Research farm near Tribune, Kansas (38^{0}27.56.03” N, 101^{0}45’40.17” W, elevation: 1100 m). Temperature and precipitation was assessed for each location from the webpage www.accuweather.com.

Information for growing degree days (GDD) and a spring barley development model were obtained from the North Dakota Agricultural Weather Center (Enz and Vasey 2005). Growing degree days were calculated with the formula GDD = ((Daily maximum air temperature + daily minimum air temperature)/2) – minimum threshold temperature. The model was used to calculate a minimum temperature threshold for barley development of 16 °C and a minimum accumulated GDD to start flag leaf emergence 1,218 GDD.

Experimental Design and Treatments

The experimental design of the trials was a split plot design with two main plot treatments (planting dates) replicated eight times in a randomized complete block design (Appendix A). The early planting date plots were sown on March 21-23, 2007, March 11-13, 2008, and March 15-19, 2009. Plants in the normal planting plots were sown on April 22, 2007, April 12-13, 2008, and April 13 and April 19, 2009. Within each planting date, four split-plot treatments (varieties) were randomized. These treatments included the RWA-resistant barley cultivars Stoneham and Sidney, and the susceptible cultivar Otis under thiamethoxam-protected and unprotected regimes (Otis_p and Otis, respectively). Individual subplots were 9.15 m wide and

12.2 m long. Alleys were treated with glyphosate to eliminate plants harboring background aphid populations developing in these areas. The pedigree for the RWA-resistant variety Sidney is (Otis x STARS 9301B) and for Stoneham is (Otis x 9577B). Previous research indicates that resistance in Stoneham is linked to two quantitative trait loci (QTLs), one on the short arm of chromosome 1H and one on the long arm of chromosome 3H. In the case of Sidney, resistance is linked to the two QTLs associated with Stoneham plus another on chromosome 2H (Nieto-Lopez and Blake 1994, Mornhinweg et al. 2006, Bregitzer et al. 2008, Mittal et al. 2008, Mittal et al. 2009).

Aphid Sampling

During the growing season, aphids were collected during the barley tillering stage, jointing stage, boot stage, and early dough stage. In the early-planted plots, these stages occur in early May, mid-May, early June and late June, respectively, and in the normal-planted plots, these stages occur in mid-May, early June, late June, and early July. In order to obtain aphid samples, 25 tillers were randomly collected in each split-plot treatment and inspected for the presence of RWA. Tillers were bagged in self-sealing plastic bags and returned to the ARDEC, Fort Collins, Colorado facilities and placed in Berlese funnels for 24 hours. The samples then were cleaned with water, preserved in 80% alcohol, and the numbers of aphids were determined.

Data Analysis

RWA obtained from plots at each location were counted at the end of the barley growing season. The total number of aphids for each subplot (varietal treatment) was obtained by totaling the RWA aphid counts across the four sampling dates, and the average of this total was obtained by averaging the number of RWA in the eight replications collected for early and normal planted plots. Aphid population data were transformed (Log [number of aphids + 1]) to reduce the dispersion of the data and to assume normality of the data. Data were then analyzed using the SAS PROC MIXED procedure (SAS, 2003), which accounted for random effects plots, blocks and their interactions within locations, planting dates, and varieties.

The analysis of variance evaluated individual effects such as location, planting date, and variety. The effect of planting dates and varieties by location was determined using the command SLICE within the PROC MIXED command (SAS 2003). Least significant differences (LSMEANS) between mean treatments were determined using a t-test for pairwise comparisons with pr<|t| = 0.05.

In order to establish differences in varietal treatments, a multiple comparison test using orthogonal contrasts (SAS 2003) was used to determine differences in RWA populations between resistant varieties (Sidney and Stoneham combined effect) vs. the susceptible variety, Otis. Also, the orthogonal contrast was used to evaluate the differences between RWA populations on Otis-p vs. resistant varieties; and finally differences in RWA populations between Otis and Otis_p varietal treatments.

Results and Discussion

Effect of Location on RWA populations

A complete analysis of variance for RWA populations collected during 2007, 2008 and 2009 (Table 2.1) indicated that at each location, differences in RWA populations were highly significant in each of the three years of research (Figs. 2.1, 2.2, 2.3). In general, higher populations occurred at Fort Collins, Colorado than at Tribune, Kansas or Sidney, Nebraska in 2007 and 2008. Also, highly significant differences were observed between the three experimental sites in 2008 and 2009, with the lowest RWA populations observed in Nebraska in 2008 and 2009. In 2009, RWA populations at Tribune were 10-fold greater than at Fort Collins and 66-fold greater than at Sidney (Fig. 2.3), and 45-fold greater than populations at Tribune in 2008 (Fig. 2.2). The reduced population at Fort Collins in 2008 was likely related to the loss of one-third of the experimental area after a tornado touched the ground near the ARDEC facilities. This event likely explains the high variation in RWA population counts and the high dispersion of data at this location in 2008. Differences in climatic profiles at each location are illustrated in Figs. 2.1, 2.2 and 2.3.

Mean RWA populations at Fort Collins were likely higher at Tribune and Sidney in 2007 and 2008 (Figs. 2.1 and 2.2) because of the ideal temperature range (18°-19°C) for RWA development and the intermediate level of precipitation that occurred at this site (~70 mm.). The temperature range (23°C) was also favorable at Tribune, but cumulative precipitation throughout the growing season was much lower, ranging from only 22 to 33 mm. In general, wheat and barley plants respond to water stress by accelerated development (McMaster and Wilhelm 2003, McMaster et al. 2005), which may explain why RWA populations were low at Tribune during drought periods. More rapidly developing plants are not synchronized with RWA colonization and RWA populations are correspondingly reduced.

A very favorable temperature range (18°-21°C) for RWA development occurred at Sidney as well, but in contrast to Tribune and Fort Collins, the ~200 mm of precipitation at Sidney was almost three times that at Fort Collins, and almost seven times that at Tribune. Interestingly, RWA populations increased dramatically at Tribune in 2009 compared to Sidney and Fort Collins (Fig 2.3). A plausible explanation for this difference was an increase in cumulative precipitation (155 mm) at Tribune that was seven times greater than 2007 or 2008, and a stable mean temperature of 23°C that was higher compared to Fort Collins and Sidney. In contrast, populations at Sidney in 2009 were generally lower due to ~392 mm of rainfall, which was almost 3 times that occurring at Tribune and 2 times that occurring at Fort Collins. Temperature, precipitation, and mean RWA population at each location across years are shown in Fig. 2.4, using common scales for precipitation and RWA populations at all three locations.

Results of Hughes (1988) and Hughes and Maywald (1990) indicate that areas with high rainfall are not favorable for RWA infestation, and are consistent with the low RWA populations observed in Nebraska during the three years of research. Hughes (1988) and Hughes and Maywald (1990) also suggested that high RWA populations occur in warm, dry barley and wheat production areas in South Africa and the United States, with low soil moisture (up to 60 mm), and a mean of 300 mm of rainfall. However, this information does not entirely coincide with the RWA population dynamics observed at Tribune in 2009, where precipitation was ~155 mm. Mean temperatures in North Central Colorado, Western Kansas, and Western Nebraska ranged from 18-23°C, and were similar to the 16-21°C temperature range which was reported to be ideal for RWA nymphal development (Hein et al. 1988, Michaud and Sloderbeck 2005). Hughes (1988) also commented that RWA development and reproduction rates are linearly related to temperature between 2°C and 25°C, and that reproduction and development do not occur below or above those limits. Since, temperature ranges in each location were ideal for RWA development, excess precipitation appeared to have been the major limiting factor in RWA development. Climatic conditions at Fort Collins, Colorado were fair for development, with a good temperature range but high precipitation. The temperature and precipitation levels at Tribune, Kansas provided ideal climatic conditions in 2009. Climatic conditions at Sidney, Nebraska were the least favorable for RWA development because of excessive precipitation. In order to develop an improved idea of the interactions between climatic factors (e. g. temperature and precipitation) and RWA populations, the effects of temperature and precipitation will need to be assessed in controlled environment experiments.

RWA Population Differences from Multiple Effect Interactions

Differences in RWA populations by planting date and location. Due to the differences in RWA populations in each location, the planting date and variety factors were evaluated for each site individually. When assessed by location and planting date, consistently higher RWA population densities were found in normal- planted plots in Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska than in early-planted plots. However, due to high fluctuations in populations, these differences were significant only for Fort Collins and Tribune in 2007 (Fig. 2.1), and Fort Collins in 2009 (Fig. 2.3). At Sidney, very low RWA populations were observed during the three years of data collection, even on plants grown under normal planted regimes. As mentioned previously, mean RWA populations at Fort Collins in 2008 were unusually uneven, likely due to the loss of one-third of the experimental area as a result of a tornado.

A plausible explanation for the differences in populations in early- and normal- planted plots was the synchronization of RWA colonization with the vegetative stages of barley growth. As explained by Hendrix (2002), barley has a determinate growth habit, and all vegetative growth is complete at flowering. During vegetative growth, photoassimilates are loaded from the phloem into vegetative cells via the apoplast. However, as flowering begins, seeds enlarge and photoassimilates are partitioned and remobilized from stems to developing embryos and/or endosperm. Thus, the most suitable barley growth stages for RWA feeding, development, and reproduction are the tillering and stem extension stages, when plant resources are invested in vegetative growth. Once the flag leaf emerges, nutrients are translocated to developing seeds and leaf senescence begins.

The more rapid growth and development of plants in early-planted plots than plants in normal-planted plots was likely an additional important factor determining RWA colonization. Differences between the date of barley flag leaf emergence of plants in early- and normal-planted plots at Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska are shown in

Figs. 2.5B, 2.6B and 2.7B. In theory, reduced RWA populations developing in early-planted plots likely occur due to the reduced amount of time that early- planted plots are exposed to alate RWA individuals before flag leaf emergence, and alates colonizing plants in normal planted plots benefited from a greater amount of time for establishment, feeding and population development. In reality, results at Fort Collins supported this scenario, and early- planted plots produced lower RWA populations than normal- planted plots, with the exception of 2008, when the tornado occurred. At Tribune and Sidney however, results were quite different. Drought in 2007 and 2008 at Tribune adversely affected barley growth and RWA population development at both planting dates, while favorable climatic conditions in 2009 allowed large RWA population development and yielded no significant differences in RWA populations between early- or normal planted plots. During all three years at Sidney, small populations developed in both early- and normal-planted plots and differences were likely prevented due to heavy pecipitation. In general, the current results indicate that planting spring barley as early as the first week in March is a strategy that may help better manage RWA populations, because the synchrony between the arrival of RWA alates and suitable vegetative stages in the plant is disrupted.

Differences in mean RWA populations by varietal treatment in each location. The mean RWA populations occurring on resistant and susceptible barley varieties at each research site in 2007, 2008 and 2009 are shown in Figs. 2.8, 2.9 and 2.10, respectively. At Fort Collins, the highest populations occurred on the susceptible variety Otis, followed by those on Sidney and Stoneham. The lowest populations occurred on Otis plants treated with insecticide. As indicated previously, very low populations occurred at Tribune in 2007 and 2008 due to drought, and very high populations occurred in 2009 that were independent of varietal treatment. At Sidney, populations were very low, but those on Otis plants were significantly greater than populations on any other varieties. Significant differences in RWA population levels between varieties at each site and in each year are summarized in Table 2.2.

RWA resistance in Sidney (Otis x STARS 9301B) and Stoneham (Otis x STARS 9577B) has previously been demonstrated by Mornhinweg et al. (1995), Mornhinweg et al. (1999) and Bregitzer et al. (2003). Bregitzer et al. (2003) showed that RWA populations were similar on susceptible and resistant varieties under greenhouse conditions but different under field conditions, and suggested that differences in the field were because resistant plants do not roll their leaves after infestation, which subjects RWA to mortality from natural enemies and abiotic factors such as wind or rain. A more recent study, by Murugan et al. (2010) demonstrated that Sidney and Stoneham exhibit moderate levels of antibiosis (reduced intrinsic rate of increase) and tolerance (reduced root and shoot dry weight loss) to RWA biotypes 1 and 2. In the present study, differences in RWA populations on different varieties were highly dependent on climate at each location. In general, Sidney and Stoneham supported intermediate to low levels of RWA infestation compared with the susceptible variety Otis at Fort Collins, Colorado. Significant differences in populations were also observed between Otis and the resistant varieties at Sidney and in 2007 and 2009, and at Tribune in 2007. Thus, results of the present study indicate that if climatic conditions are favorable for plant development, RWA resistance is maintained, but if climatic conditions are adverse for plant development, RWA-resistant varieties may not withstand RWA attack.

Differences between varietal treatments were also subjected to orthogonal contrast analysis (Table 2.3), and in general, there was a similar trend in 2007, 2008 and 2009. Otis, the susceptible variety, was significantly different than Otis_p, suggesting that seed treatment is effective in reducing RWA populations at each research location. There were also differences between Otis and the resistant varieties, which meant that the tolerance present in the RWA-resistant varieties helped reduce RWA infestation. Finally, there were no significant differences in RWA populations on Otis protected plants and plants of RWA-resistant varieties. This information is of great importance because farmers can be assured that tolerant varieties are as effective as the use of seed treatment as a means to control RWA populations.

Differences on mean RWA populations by varieties within planting dates in each location. Differences in RWA mean populations for varietal treatments within planting dates at Fort Collins, Sidney and Tribune in 2007, 2008 and 2009 are presented in Figs. 2.11, 2.12, and 2.13, respectively. At Fort Collins in 2007, RWA populations were significantly lower on plants of Otis, Otis_p, Sidney, and Stoneham planted early compared to those planted at the normal time (Fig. 2.11). The same differences were apparent at Tribune, Kansas for Otis and Otis_p, but there were no significant planting date differences for Sidney and Stoneham. At the Sidney, Nebraska site, planting date had no effect on RWA populations for any variety except Stoneham, where significantly fewer RWA were produced on early planted plants than on normal planted plants. In 2008, there were no significant varietal differences in RWA populations based on planting dates at Fort Collins, Sidney or Tribune (Fig. 2.12). However, populations produced on

Otis plants at both planting dates were significantly higher than infestations on Otis_p, Sidney, or Stoneham.

In 2009, significant differences in planting dates were observed for Otis at Fort Collins. Otis grown in the normal planting dates plots were significantly more infested that Otis grown early in the season. Intermediate infestation levels were observed for Sidney and Stoneham grown in the normal planting plots and lowest infestation levels were observed for Otis_p in the two planting date's plots and for Sidney and Stoneham grown in the early planting date plots. A very high infestation was observed at Tribune in 2009 when compared with average infestation levels at Fort Collins or Sidney. No differences in planting dates or varietal treatments were determined due to the high infestation levels sustained in all the treatments. Very low infestation levels were observed at Sidney in 2009, when compared with Fort Collins or Tribune. Significant differences between planting dates were observed for Otis, but no differences by planting dates were obtained for the other varietal treatments. Intermediate infestation levels were observed for Sidney, and the lowest infestation levels were observed for Otis_p, and Stoneham. One explanation for this result could be the fact that in general RWA do not achieve pest status and remains as an innocuous resident in areas with normal rainfall, as in the Mediterranean region. For this particular region, this insect only becomes of economic importance in years with prolonged drought (Miller et al. 1993) as seen at Tribune, Kansas in 2009. In general, the use of cultural practices such as planting dates, the use of treated seed with insecticide, and the selection of tolerant barley varieties well-adapted to specific growing conditions can be used to manage RWA populations, as previously suggested by Peairs (1998).

Conclusions

During the three years of the present study, RWA infestation levels were highly variable between locations, and infestations at Tribune, Kansas were the most variable. Infestations were less variable at Fort Collins, Colorado across years, and infestations at Sidney, Nebraska were the lowest and least variable of the three locations. As discussed previously, the very different climatic conditions at each location are the most likely explanation for high variation in RWA infestation levels. RWA outbreaks occurred in areas with high temperatures and medium levels of precipitation, in this particular case, the Tribune, Kansas field site.

The more rapid growth and development of early planted plots than normal planted plots appears to be an important factor in limiting RWA population development. In general, planting date differences depend on the accumulation of degree days, with early-planted plots providing a a smaller window for RWA colonization than normal-planted plots. However, this observation would also depend upon the specific climatic conditions offered by each location. My results indicate that early planting helped reduce RWA infestations at Fort Collins in each of the three years of research. Early planting appears to be a useful cultural control strategy in western Kansas, but further research is necessary to evaluate how drought stress affects the performance of both plants and aphids.

The low to intermediate levels of RWA infestation on Sidney and Stoneham barley may be a product of either tolerance and/or antibiosis resistance. However, the similarity in RWA populations on plants of Sidney and Stoneham to those of susceptible Otis plants protected by insecticide seed treatment suggests that antibiosis resistance is acting to reduce RWA populations.

In a broad sense, an additive combined effect of early planting date and the RWA-resistant variety was observed in the three years of research in the central High Plains area of barley production. The RWA-resistant barley varieties Sidney and Stoneham, grown early in the season, produced the lowest RWA infestation levels when compared with susceptible Otis plants. These two integrated pest management strategies can contribute to significant reductions in RWA infestations on barley produced in east central for Colorado, western Kansas, and western Nebraska.

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Tables and Figures

Table 2.1 Analysis of variance for RWA populations at Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska in 2007, 2008 and 2009.

Effect	Loc	DF	2007		2008		2009	
			F value	Pr > F	F value	Pr > F	F value	Pr > F
Loc		2	11.68	<.0001	12.07	<.0001	190.58	<.0001
Pltdate	Across locations	1	70.87	<.0001	5.94	0.0196	6.57	0.0181
Loc*Pltdate	Across locations	2	10.09	0.0001	1.25	0.2988	1.48	0.2509
Loc*Pltdate	CO	1	59.94	<.0001	5.89	0.0200	6.54	0.0183
Loc*Pltdate	KS	1	29.01	<.0001	2.49	0.1228	0.03	0.8728
Loc*Pltdate	NE	1	2.11	0.1499	0.05	0.8313	2.96	0.1000
Variety	Across locations	3	20.00	<.0001	3.60	0.0185	11.99	<.0001
Loc*Variety	Across locations	6	2.52	0.0271	3.08	0.0109	4.86	0.0014
Loc*Variety	CO	3	15.84	<.0001	8.83	<.0001	15.49	<.0001
Loc*Variety	KS	3	3.03	0.0339	0.48	0.6968	1.84	0.1609
Loc*Variety	NE	3	6.18	0.0008	0.46	0.7143	4.38	0.0112
Pltdate*variety	Across locations	3	2.33	0.0802	0.46	0.7100	4.45	0.0057
Loc*Pltdate*Variety	Across locations	6	7.74	<.0001	1.40	0.2284	0.82	0.5550
Loc*Pltdate*Variety	CO	7	18.09	<.0001	5.11	0.0001	8.89	<.0001
Loc*Pltdate*Variety	KS	7	9.17	<.0001	1.47	0.1963	1.00	0.4470
Loc*Pltdate*Variety	NE	7	4.13	0.0005	0.21	0.9809	3.39	0.0047

Analysis of variance determined with Proc MIXED, with fixed effects SE method, and degrees of freedom obtained by Satterthwaite method (See Appendix B for SAS Codes)

Total number of observations for the analysis= 192

Number of observations per location= 64, observations per planting date= 96, observations per variety= 48

Number of observations per planting date/location = 32

Number of observations per variety/location = 16

Number of observations per variety/planting date/location = 8

Table 2.2 Effect of barley varieties on mean RWA populations across planting dates at Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska in 2007, 2008, and 2009

Location	Variety	2007				2008				2009			
		Mean ± S.E.[a]				Mean ± S.E.				Mean ± S.E.			
Colorado	Otis	57.9	±	11.616	a	39.6	±	14.243	a	55.1	±	11.492	a
	Otis_p	6. 6	±	4.193	c	3.6	±	1.700	b	7.9	±	1.368	c
	Sidney	16.8	±	6.623	b	2.8	±	1.336	b	17.7	±	3.100	b
	Stoneham	16.4	±	4.140	b	3.9	±	1.595	b	20.4	±	3.702	b
Kansas	Otis	18.6	±	6.525	a	5.6	±	3.257	a	328.2	±	53.785	a
	Otis_p	5.3	±	1.680	b	12.2	±	9.410	a	316.4	±	50.534	a
	Sidney	3.0	±	0.983	b	3.8	±	3.102	a	193.1	±	31.332	a
	Stoneham	2.6	±	0.769	b	1.7	±	0.892	a	240.6	±	39.692	a
Nebraska	Otis	18.0	±	3.458	a	0.1	±	0.085	a	7.2	±	2.646	a
	Otis_p	4.6	±	1.522	b	0.0	±	0.000	a	1.6	±	0.397	b
	Sidney	4.2	±	1.156	b	0.1	±	0.060	a	4.7	±	2.314	b
	Stoneham	5.4	±	1.519	b	3.6	±	3.023	a	1.6	±	0.446	b

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam regime, Resistant varieties = Sidney and Stoneham.

[a] Means followed by the same letter within each column by location are not significantly different (pr<|t| = 0.05)

Table 2.3 Orthogonal contrasts obtained in order to determine barley varietal differences in mean RWA populations at Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska in 2007, 2008 and 2009.

Contrast	DF	2007		2008		2009	
		F	**Pr > F**	**F**	**Pr > F**	**F**	**Pr > F**
Otis versus Otis_p	1	46.53	<.0001**	5.75	0.0196 *	31.07	<.0001 **
Otis versus Resistant varieties	1	48.09	<.0001**	9.57	0.0030 **	25.20	<.0001 **
Otis_p vs. Resistant varieties	1	0.89	0.3491 n. s.	0.11	0.7464 n. s	2.01	0.1669 n. s.

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam regime, Resistant varieties = Combined effect of Sidney and Stoneham.

Orthogonal contrast determined with Proc MIXED (SAS 2003)

** Significant at 1%,* significant at 5%, n. s. = non significant differences

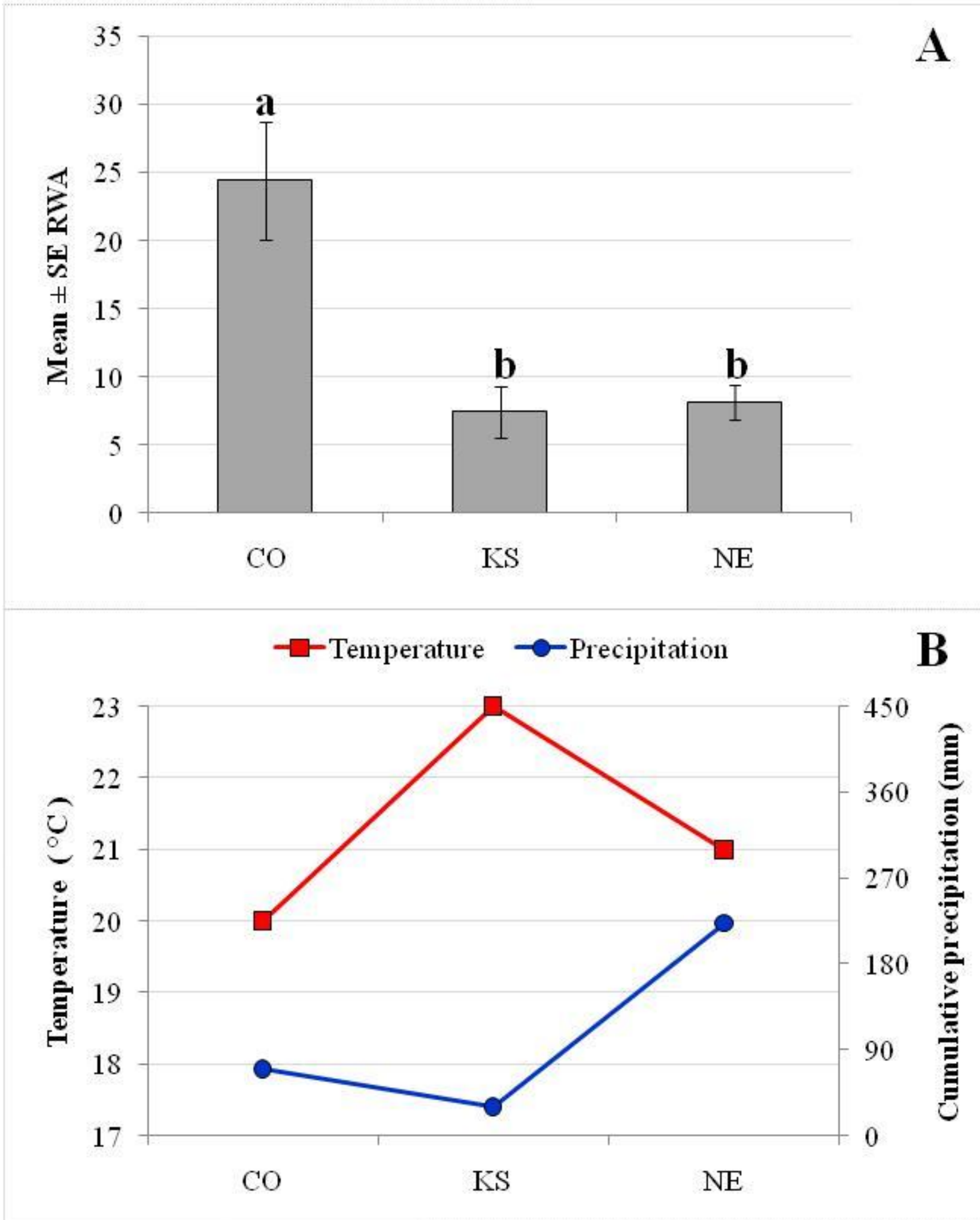

Fig. 2.1 A) Mean RWA populations and B) Mean maximum temperature and cumulative precipitation at Fort Collins, Colorado (CO), Tribune, Kansas (KS) and Sidney, Nebraska (NE), in the 2007 growing season from March to early July.

Significant differences with pr<|t| = 0.05

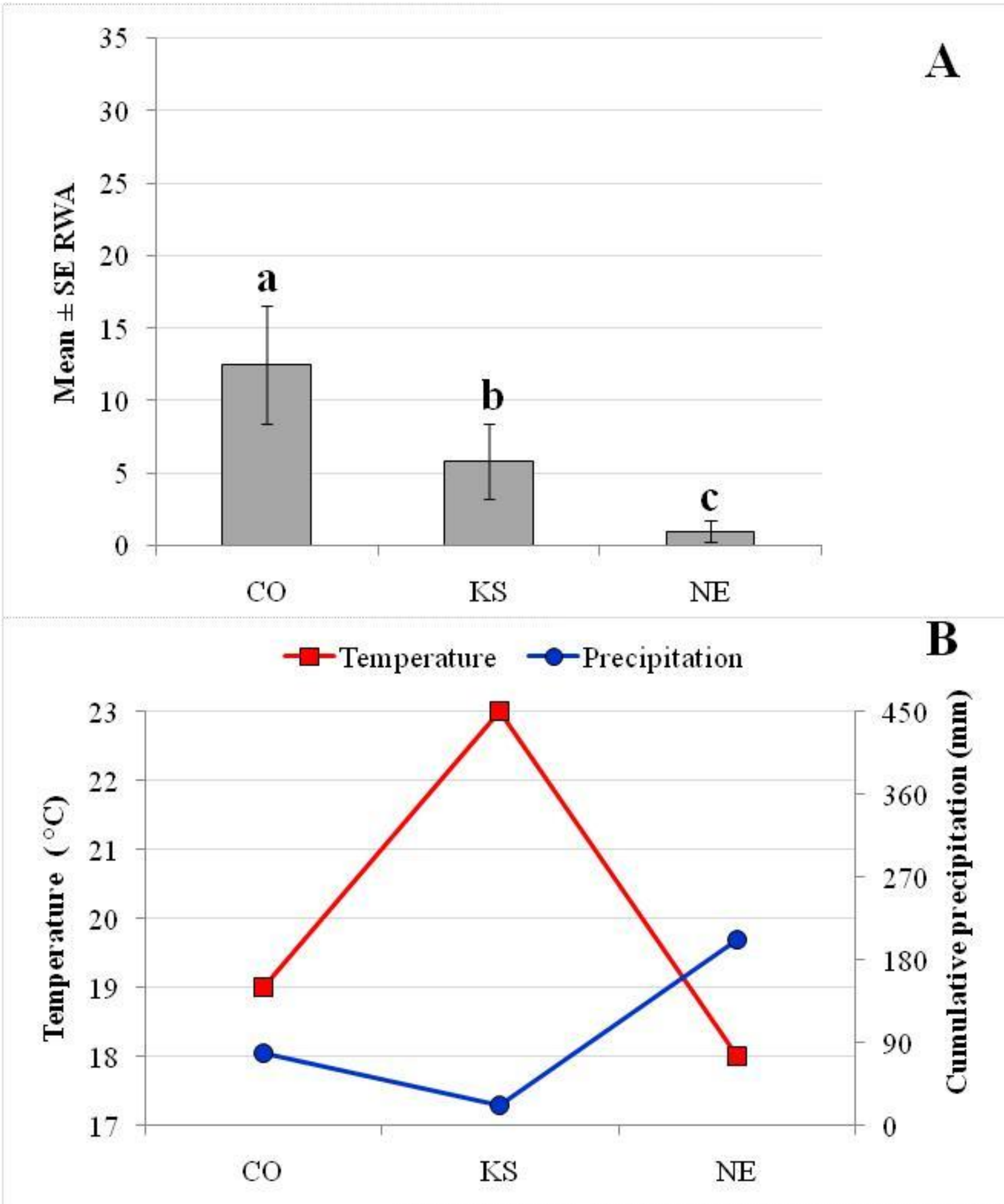

Fig. 2.2 A) Mean RWA populations and B) Mean maximum temperature and cumulative precipitation at Fort Collins, Colorado (CO), Tribune, Kansas (KS) and Sidney, Nebraska (NE), in the 2008 growing season from March to early July.

Significant differences with pr<|t| = 0.05

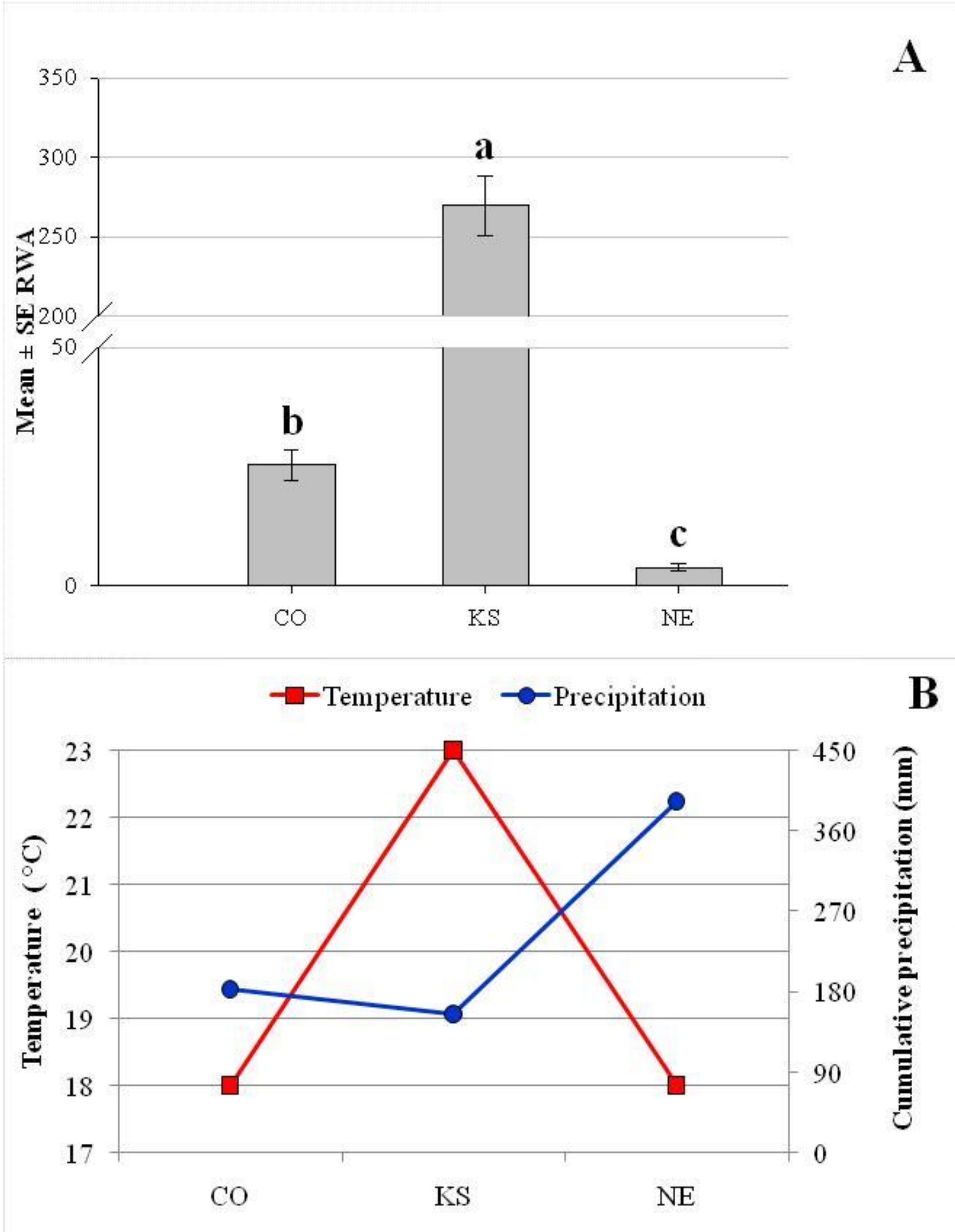

Fig. 2.3 A) Mean RWA populations and B) Mean maximum temperature and cumulative precipitation at Fort Collins, Colorado (CO), Tribune, Kansas (KS) and Sidney, Nebraska (NE), in the 2009 growing season from March to early July.

Significant differences with pr<|t| = 0.05

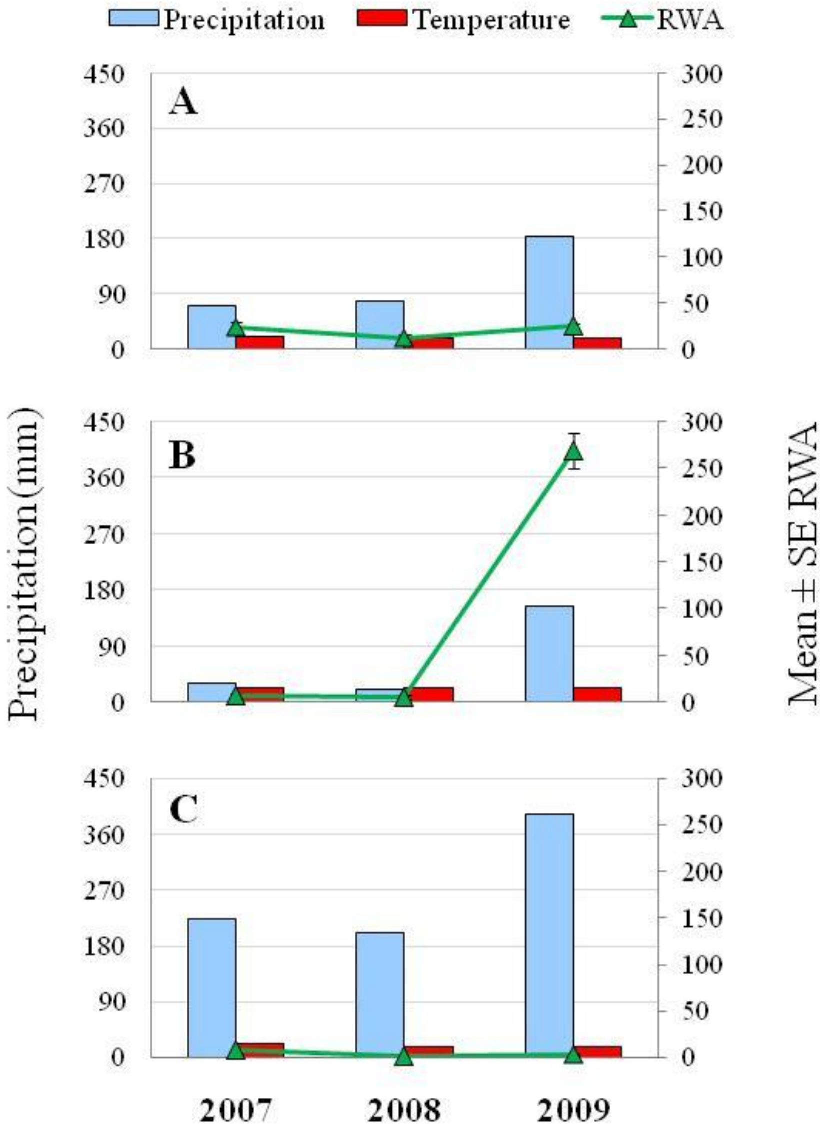

Fig. 2.4 Cumulative precipitation, mean maximum temperature, and mean RWA population at A) Fort Collins, Colorado, B) Tribune, Kansas, and C) Sidney, Nebraska in 2007-2009.

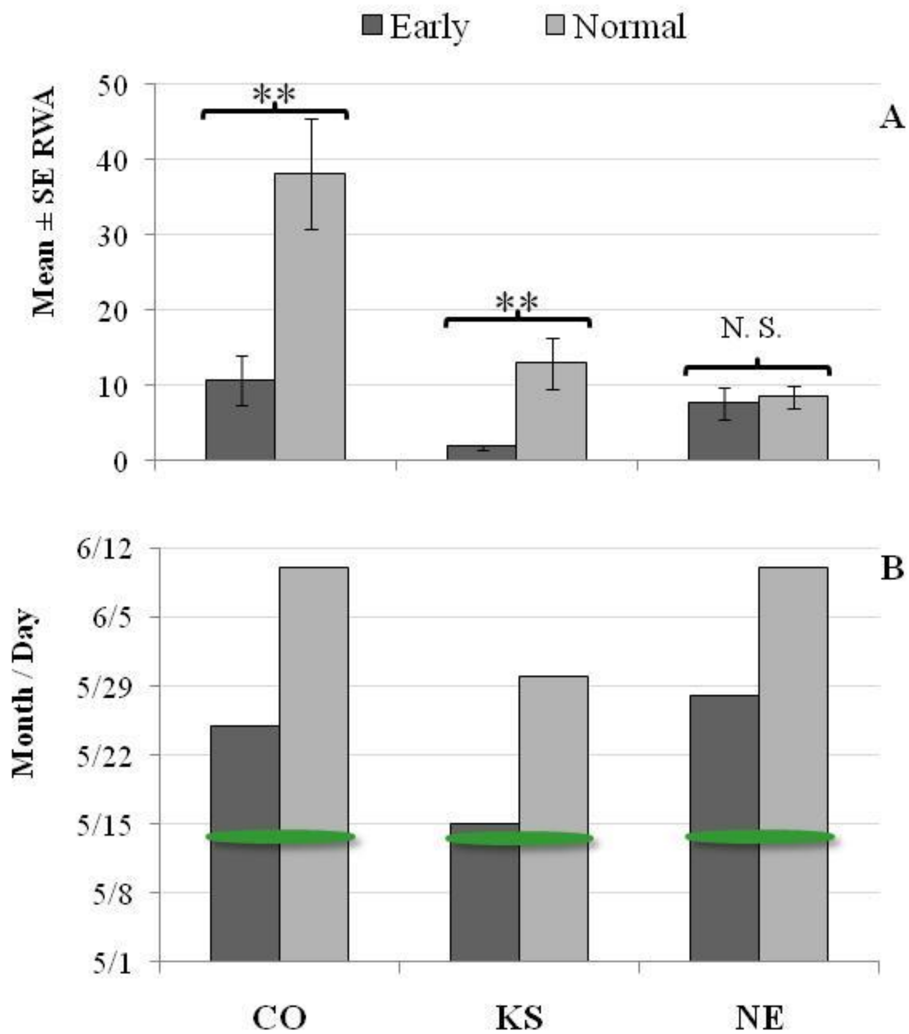

Fig. 2.5 Effect of location and barley planting date on A) RWA mean populations, and B) date of flag leaf emergence at Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska in 2007. Green area = Date of first recorded RWA collection in the field.

Pair-wise comparisons for each location by year (t-test)

** Significant at 1%,* significant at 5%, n. s. = non significant differences

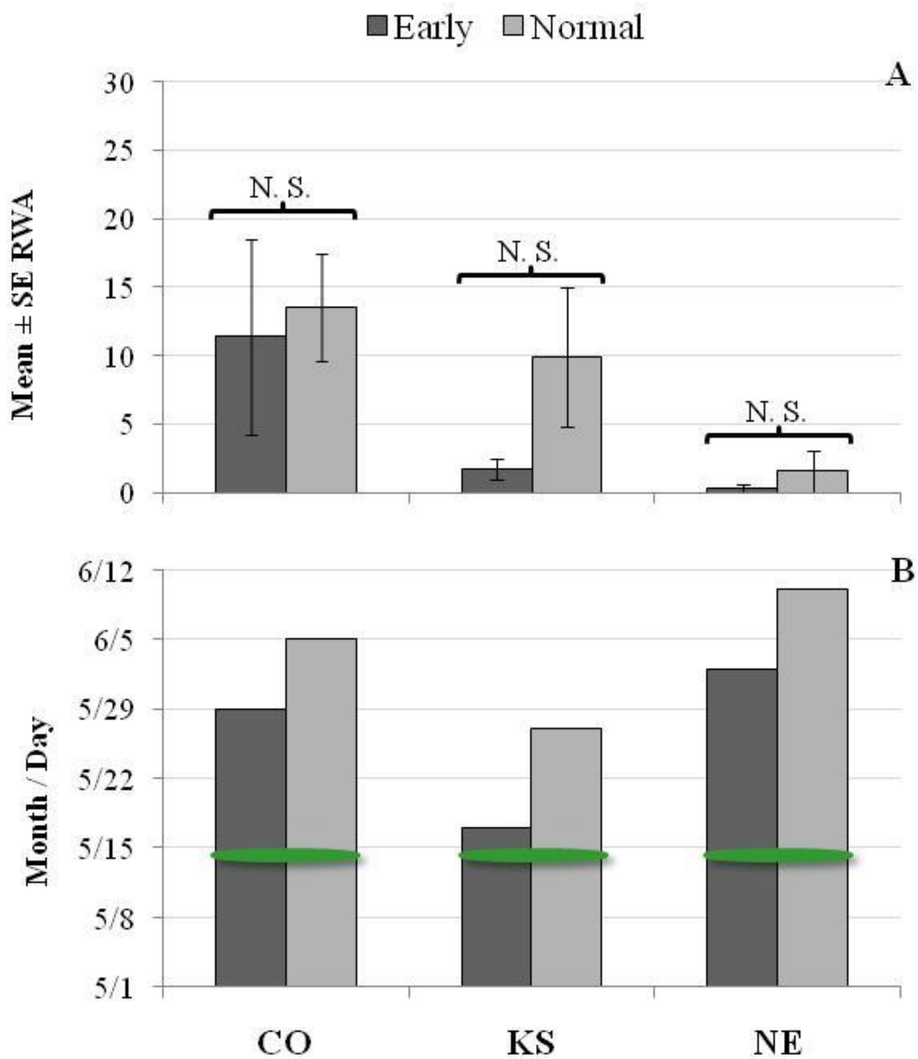

Fig. 2.6 Effect of location and barley planting date on A) RWA mean populations, and B) date of flag leaf emergence at Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska in 2008. Green area = Date of first recorded RWA collection in the field.

Pair-wise comparisons for each location by year (t-test)

** Significant at 1%,* significant at 5%, n. s. = non significant differences

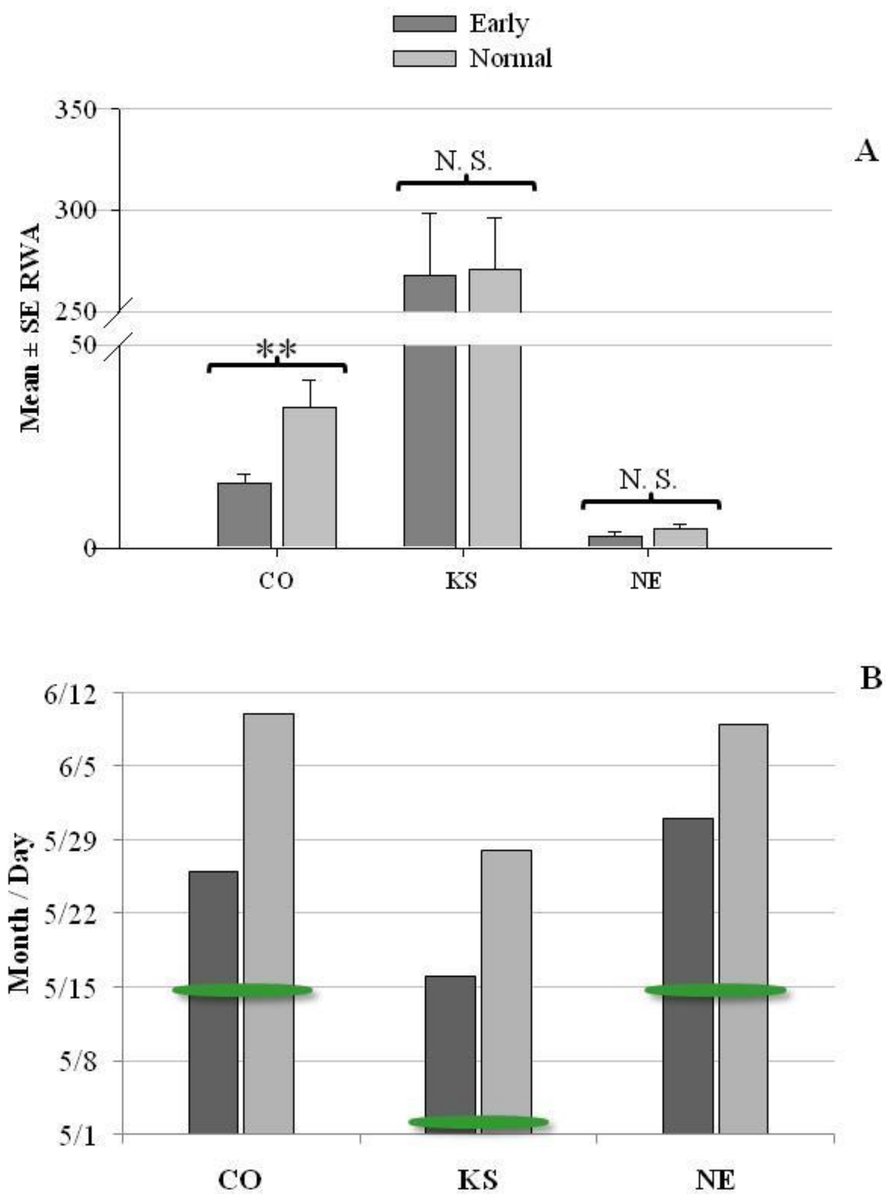

Fig. 2.7 Effect of location and barley planting date on A) RWA mean populations, and B) date of flag leaf emergence at Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska in 2008. Green area = Date of first recorded RWA collection in the field.

Pair-wise comparisons for each location by year (t-test)

** Significant at 1%,* significant at 5%, n. s. = non significant differences

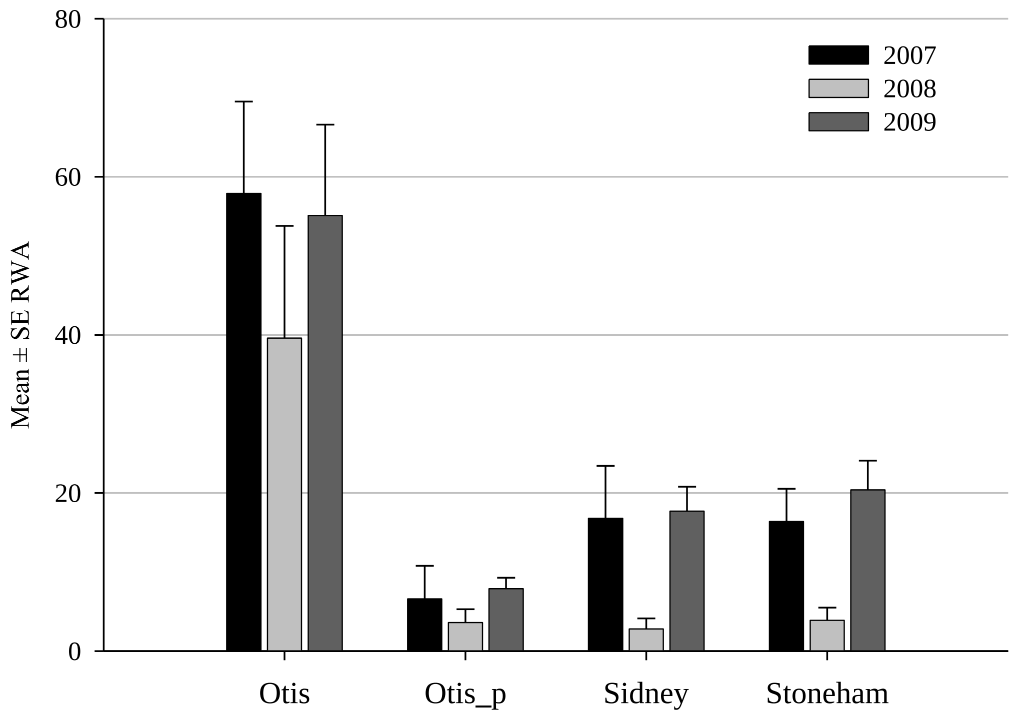

Fig. 2.8 Mean ± SE RWA populations on resistant and susceptible barley varieties at Fort Collins, Colorado 2007- 2009.

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam regime, Resistant varieties = Sidney and Stoneham.

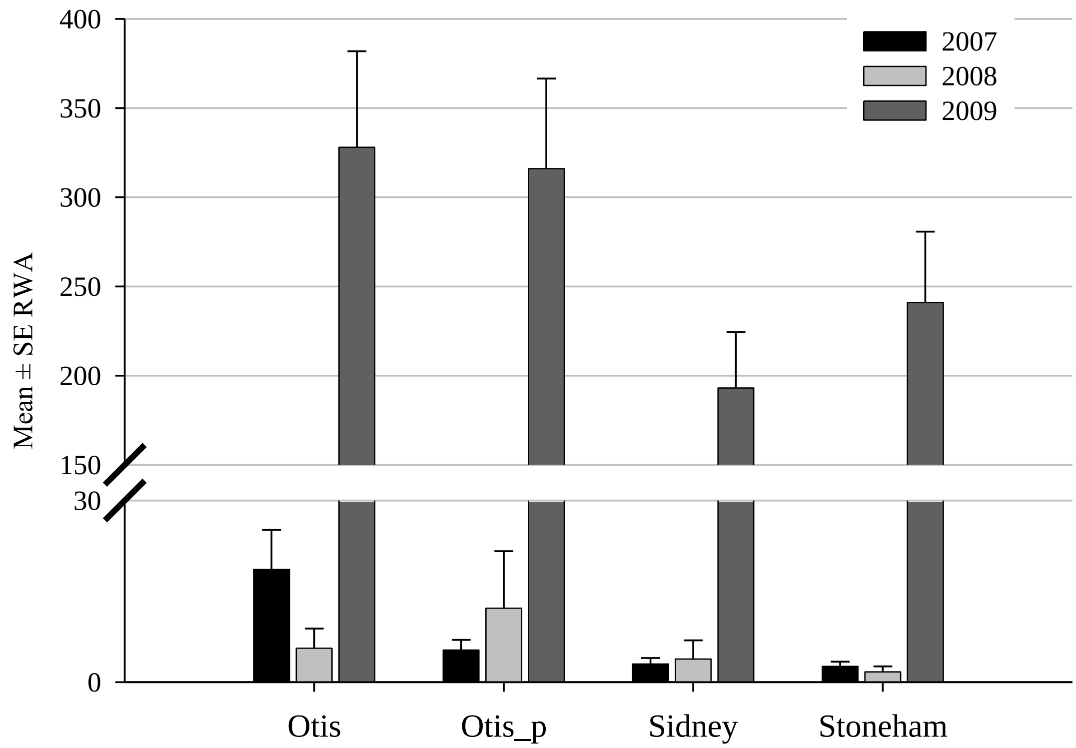

Fig. 2.9 Mean ± SE RWA populations on resistant and susceptible barley varieties at Tribune Kansas 2007- 2009.

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam regime, Resistant varieties = Sidney and Stoneham.

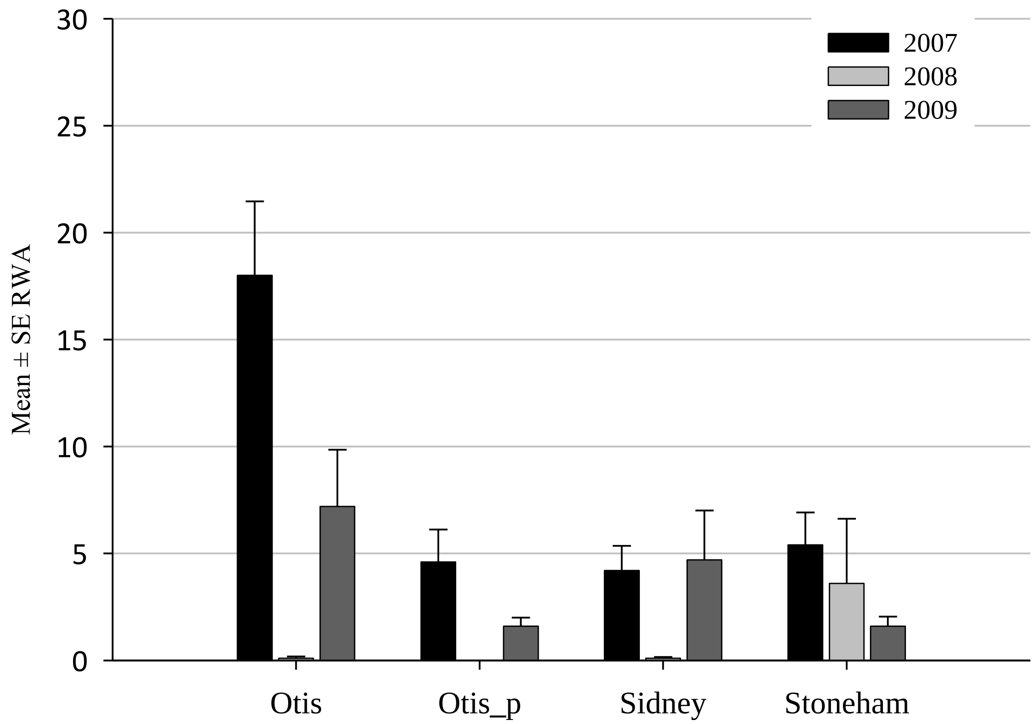

Fig. 2.10 Mean ± SE RWA populations on resistant and susceptible barley varieties at Sidney, Nebraska 2007- 2009.

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam regime, Resistant varieties = Sidney and Stoneham.

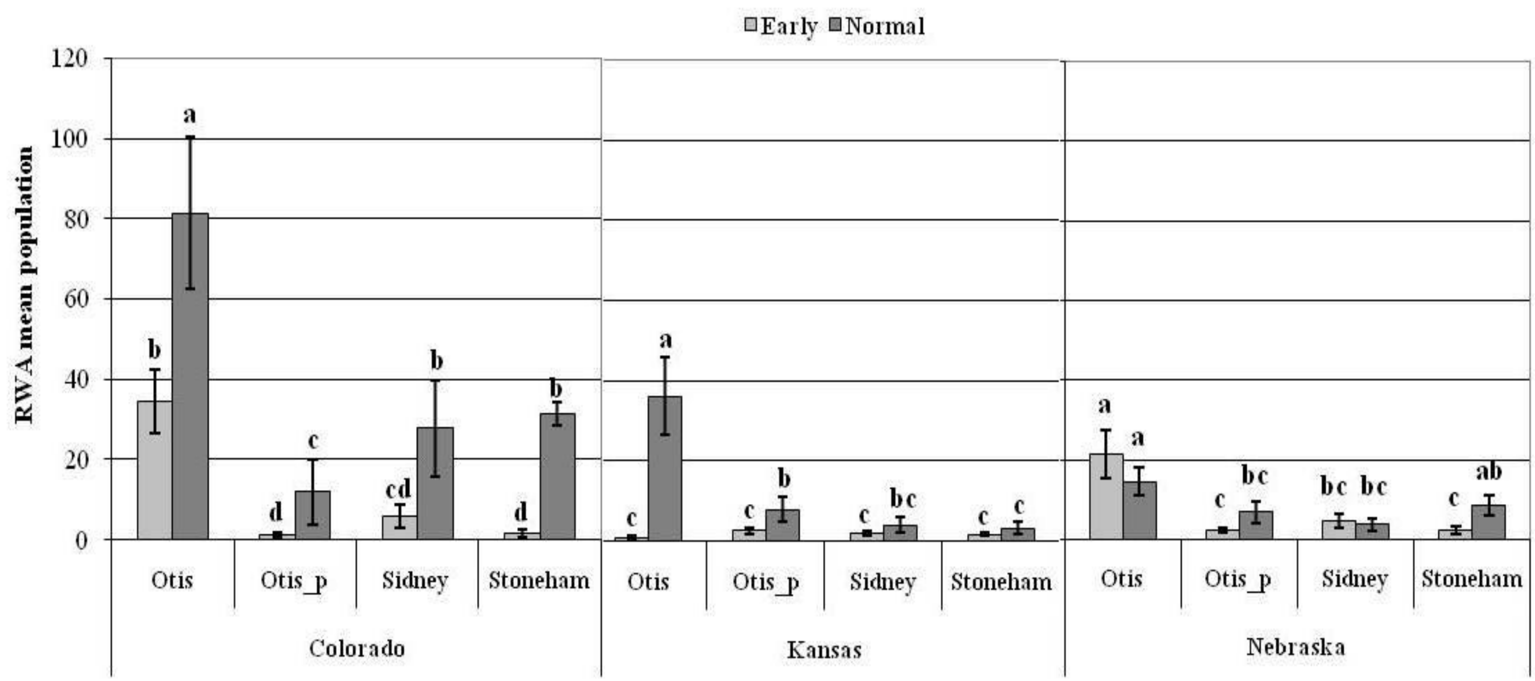

Fig. 2.11 Effect of planting date on mean RWA populations across varieties at Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska in 2007

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

Means followed by the same letter for each location are not significantly different (pr<|t| = 0.05)

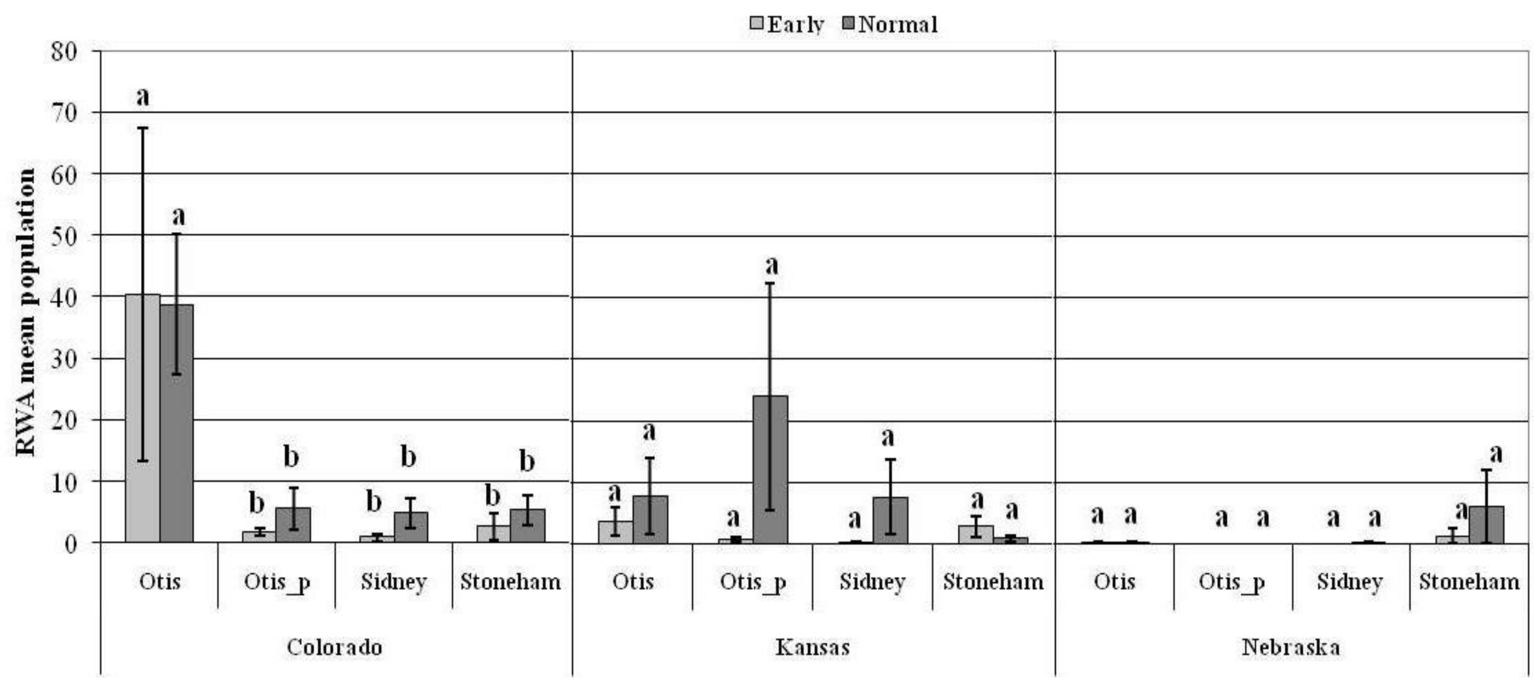

Fig. 2.12 Effect of planting date on mean RWA populations across varieties at at Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska in 2008

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

Means followed by the same letter for each location are not significantly different (pr<|t| = 0.05)

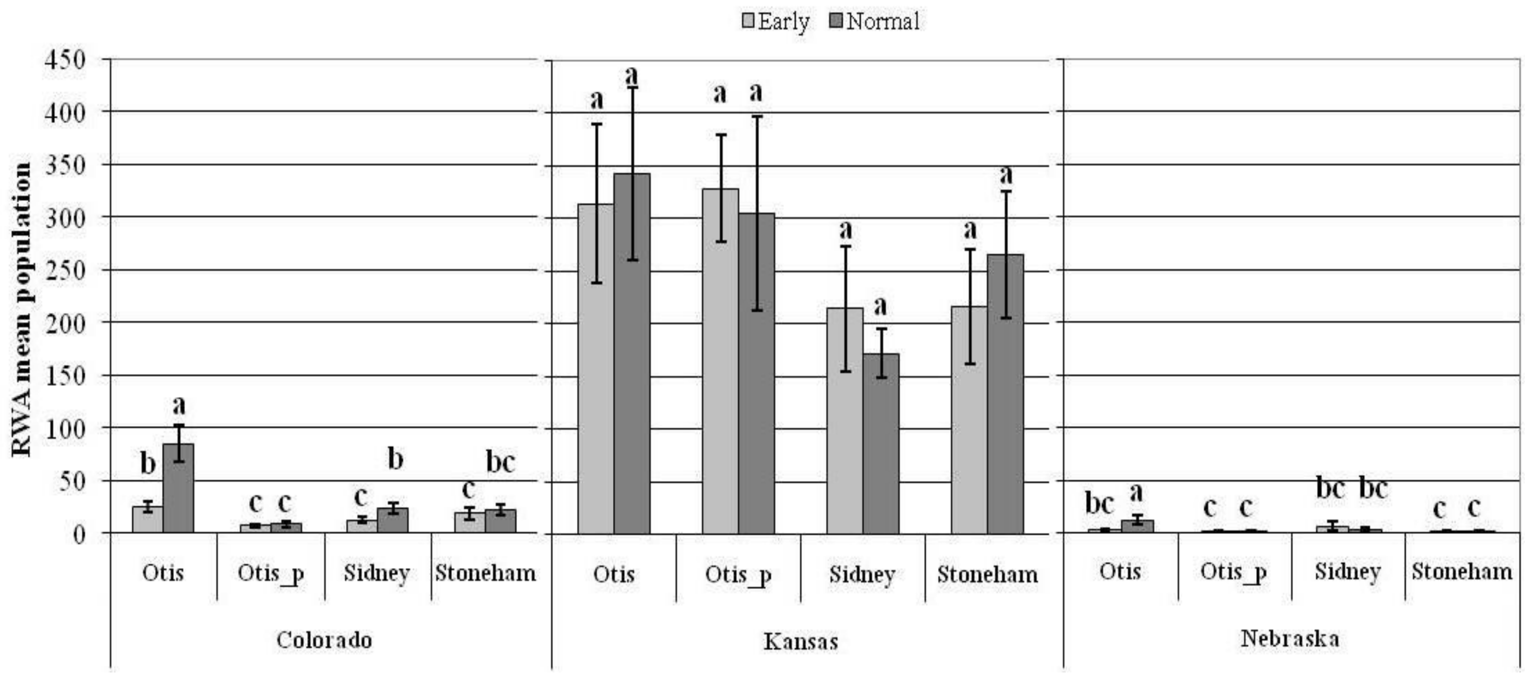

Fig. 2.13. Effect of planting date on mean RWA populations across varieties at Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska in 2009

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

Means followed by the same letter for each location are not significantly different (pr<|t| = 0.05)

CHAPTER 3 - EFFECT OF EARLY PLANTING AND RUSSIAN WHEAT APHID RESISTANT BARLEY VARIETIES ON POPULATIONS OF SMALL GRAIN CEREAL APHIDS OTHER THAN RUSSIAN WHEAT APHID

Abstract

Cereal aphids (Hemiptera: Aphididae) such as Russian wheat aphid, greenbug, bird cherry-oat aphid, and other small grain cereal aphids can be important pests by causing important economic losses in production of barley, wheat, sorghum, oats, and other small grain cereals planted in the High Plains in the U.S. Management strategies in barley against Russian wheat aphid (RWA) may potentially have different effects over cereal aphid species other than RWA. For this reason, it is necessary to analyze the changes in population dynamics of RWA and other species of cereal aphids on barley. The objective of this research was to evaluate how two integrated pest management strategies - early planting date and RWA-resistant barley variety - affected populations of cereal aphids other than RWA. Although the RWA-resistant varieties Sidney and Stoneham barley contain no known resistance to other cereal aphid species, early planted plots could provide these species an opportunity to produce greater populations that later planted plots, because of their ability to overwinter as eggs. Sidney and Stoneham were evaluated in a split-plot design with two main plot treatments (early and normal planting dates) in experimental fields at at Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska during 2007, 2008 and 2009. Four split plot variety treatments (Stoneham, Sydney, RWA-susceptible Otis, and Otis treated with thiamethoxam) were randomized within each main treatment plot. In general, populations of other cereal aphid species were higher at Fort Collins, Colorado and Tribune, Kansas than in Sidney, Nebraska, but no clear responses of other ceral aphid species to planting dates were observed. The identification of individual cereal aphid species is now necessary to assess species specific-interactions by planting date and local climatic conditions. There were no differences in the populations of other cereal aphid species on Sidney, Stoneham, and Otis at any

of the three research sites, but there were significantly fewer other cereal aphid species on Otis treated with thiamethoxam than the other varieties.

Key words: RWA-resistant barley varieties, Sidney, Stoneham, Otis, Seed treatment

Introduction

The United States is a world leader in production of many crops, including cereals such as barley, buckwheat, maize, rice, sorghum, and wheat (FAO 2010) (Table 3.1). However, the production of cereal crops can be threatened by the presence of herbivorous insects, including aphids (Poehling et al. 2007). Of the 450 aphid species recorded in crop plants, 100 are agriculturally important species and several of these species are present in economically important cereal crops (Blackman and Eastop 2000, 2007). The Great Plains grassland region of the United States has the highest concentration of damage inflicted by cereal aphids. Without doubt, the greenbug, *Schizaphis graminum* Rondani, and the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (RWA), are the most economically important aphid species in this geographic area (Brewer and Elliot 2004, Powell and Pell 2007, Michaud 2010). However, several other cereal aphids have been recorded on cereal crops in this area (Qureshi and Michaud 2005a), including: corn leaf aphid, *Rhopalosiphum maidis* (Fitch); bird cherry-oat aphid, *Rhopalosiphum padi* (L.); English grain aphid, *Sitobion avenae* (F.); western wheat aphid, *D. tritici* (Guillette); yellow sugarcane aphid, *Sipha flava* (Forbes); blackberry-cereal aphid, *Sitobion fragariae* (Walker); and rose-grass aphid, *Metopolophium dirhodum* (Walker) (Stoetzel 1987, Hein et al. 1996, Poehling et al. 2007, Michaud et al. 2010).

Attempts to develop an integrated pest management strategy (IPM) in a particular crop and against one aphid species do not necessarily imply that all aphid species hosted by the same crop will respond in the same way. One factor that affects the IPM outcome is the type of aphid generation, anholocyclic vs. holocyclic (Poehling et al. 2007). Aphids with holocyclic generations (e. g., *S. avenae*, *R. padi*, and *M. dirhodum*) will spend the winter as eggs in cereal crop production areas and with the early rise in temperatures early in the spring, the immature aphids will develop and move to young crops very early in the growing season (late April).

Cereal aphids feed on the early vegetative growth stages of cereal crop plants on photoassimilates transported by the phloem to vegetative cells (Hendrix 2002). Reproduction declines after flag leaf emergence, when nutrients begin to be translocated to developing embryos and/or endosperm (Poehling et al. 2007). In this case, altered planting date as a cultural control to reduce aphid populations would have little or no effect on holocyclic aphids. In contrast, anholocyclic aphids, in particular RWA, need to migrate from southern overwintering areas (e. g., Texas Panhandle) to more temperate areas, and the time required to reach a suitable host depends on environmental factors such as temperature and wind (Michaud 2010), and aphid flight and search abilities (Walters and Dewar 1986). Early planting dates could be more effective in this case, because the plant would avoid aphid damage at early stages of development.

A second factor affecting the outcome of aphid IPM is insect response to host plant resistant traits. If resistance is conferred by tolerance, it is commonly assumed that the fitness of herbivorous insects and their natural enemies will be unaffected (Brewer and Elliot 2004). If resistance is based on antibiosis, deleterious effects on the biology and reproduction are expected in the target insect pest (Smith 2005). However, the antibiosis may not adversely affect other pest species, and secondary pest outbreaks could be expected. This outcome is an important limitation for the use of antibiosis resistance (Pedigo and Rice 2006).

As suggested above, RWA management strategies in barley may potentially have effects on cereal aphid species other than RWA. For this reason, it is necessary to analyze the changes in population dynamics of RWA and other species of cereal aphids on barley. However, a limitation to this practical question is the morphological identification of aphids from field samples. There are no problems with morphological identification of RWA, because it is the only aphid with a “two-tail” supracaudal process. Problems in proper identification of other cereal aphid species arise, as suggested by Blackman and Eastop (2007), when continuous morphological variation is observed in several aphid species. Aphids often exhibit high plasticity of phenotype, which is highly influenced by the environment. In this sense, factors such as temperature, day length, crowding, or food quality may change pigmentation and morphometric ratios making the proper identification of aphid samples from a particular crop more difficult (Blackman and Spence 1994). When aphids are placed in alcohol for storage, the characteristic color for each aphid species is lost, and the difficulties in identification are even higher.

Due to this limitation on my research, I separated non-RWA aphids in an ecological functional group that from now on will be called collectively as ‘Other cereal aphids’. The objective of this research was to evaluate how RWA-specific IPM strategies, such as early planting date regime and RWA-resistant barley varieties, affect other cereal aphid populations.

Materials and Methods

Location

The present study was conducted at three locations within the Great Plains biogeographic region during 2007, 2008, and 2009. These study sites were located at the University of Nebraska High Plains Agricultural Laboratory near Sidney, Nebraska (41^{0}12’21.91’’ N, 103^{0}00’42.72” W, elevation: 1320 m), the Colorado State University Agricultural Research, Development and Education Center (ARDEC), near Fort Collins, Colorado (40^{0}39’09.37” N, 105^{0}00’01.67” W, elevation: 1550 m), and the Kansas State University Research farm near Tribune, Kansas (38^{0}27.56.03” N, 101^{0}45’40.17” W, elevation: 1100 m).

Experimental Design and Treatments

The experimental design of the trials was a split plot design with two main plot treatments (planting dates) replicated eight times in a randomized complete block design. Early planting date plots were sown March 21-23, 2007; March 11-13, 2008; and March 15-19, 2009. Seed in the normal planting plots were sown on April 22, 2007; April 12-13, 2008, and April 13 and 19, 2009. Within each planting date, four split-plot treatments (barley varieties) were randomized. These treatments included the RWA-resistant barley cultivars Stoneham and Sidney, and the susceptible cultivar Otis under thiamethoxam-protected and unprotected regimes (Otis_p and Otis, respectively). Individual subplots were 9.15 m wide and 12.2 m long. Alleys were treated with glyphosate to eliminate plants harboring background aphid populations developing in these areas. Individual sub-plots were 9.15 m wide and 12.2 m long. Alleys were treated with glyphosate to eliminate plants harboring background aphid populations developing in these areas. The pedigree for Sidney barley is (Otis x STARS 9301B) and for Stoneham is (Otis x 9577B). Resistance in Stoneham is linked to two quantitative trait loci (QTLs), one in the short arm of chromosome 1H and one in the long arm of chromosome 3H. Resistance in Sidney is linked to the two QTLs associated with Stoneham and a different QTL on chromosome 2H (Nieto-Lopez and Blake 1994, Mornhinweg et al. 2006, Bregitzer et al. 2008, Mittal et al. 2008, Mittal et al. 2009).

Aphid Sampling

During the growing season, aphids were collected during the barley tillering stage, jointing stage, boot stage, and early dough stage. In the early-planted plots, these stages occur in early May, mid May, early June and late June, respectively, and in the normal-planted plots, these stages occur in mid-May, early June, late June, and early July. In order to obtain aphid samples, 25 tillers were randomly collected in each split-plot treatment and inspected for the presence of RWA. Tillers were bagged in self-sealing plastic bags and returned to the ARDEC, Fort Collins, Colorado facilities and placed in Berlese funnels for 24 hours. The samples then were cleaned with water, preserved in 80% alcohol, and the numbers of aphids were determined.

Data Analysis

Cereal aphids obtained from plots at each location were counted at the end of the barley growing season. The total number of aphids for each subplot (varietal treatment) was obtained by totaling the aphid counts across the four sampling dates, and the average of this total was obtained by averaging the number of RWA in the eight replications collected for early and normal planted plots. Aphid population data were transformed (Log [number of aphids + 1]) to reduce the dispersion of the data and to achieve normality of the data. Data were then analyzed using the SAS PROC MIXED procedure (SAS, 2003), which accounted for random effects plots, blocks and their interactions within locations, planting dates, and varieties.

The analysis of variance evaluated individual effects such as location, planting date, and variety. The effect of planting dates and varieties by location was determined using the command SLICE within the PROC MIXED command (SAS 2003). Least significant differences (LSMEANS) between mean treatments were determined using a t-test for pairwise comparisons with $pr<|t| = 0.05$.

To establish differences in varietal treatments, a multiple comparison test using orthogonal contrasts (SAS 2003) was used to determine differences in cereal aphid populations between resistant varieties (Sidney and Stoneham combined effect) vs. the susceptible variety, Otis. Also, the orthogonal contrast was used to evaluate the differences between cereal aphids yielded on Otis-p vs. resistant varieties; and finally differences in cereal aphid populations between Otis and Otis_p varietal treatments.

Results and Discussion

The relative abundance of Russian wheat aphids and other cereal aphids was highly variable across years, locations, planting dates, and varieties (Figs. 3.1, 3.2, 3.3). In general, RWA populations were higher than other cereal aphid populations across locations in 2007 and 2009, and were 5- to 15- fold higher at Tribune, Kansas in 2009, in comparison to other years. Interestingly, other cereal populations were higher than RWA populations in 2008. As discussed in Chapter 2 for RWA, the climatic profile for each location may have also greatly influenced cereal aphid populations. However, interspecific competition between aphids could also be a plausible explanation that accounts for such differences. Competition between *R. padi* and *S. avenae* was demonstrated in experiments conducted by Gianoli (2000). Thus, both interspecific cereal aphid competition and climatic factors likely contribute to variation in other cereal aphid populations in the present study.

The results of several studies suggest that the most important aphid species in the western High Plains are *S. graminum*, RWA and *R. padi* (Brewer and Elliot 2004, Powell and Pell 2007, Michaud 2010). Based on this information, one reason for the differences aphid abundance could be due to aphid reproductive strategy and developmental rate. RWA, as explained before, has a holocyclic reproductive strategy (parthenogenetic production of females, high rate of development) and other cereal aphids such as *S. graminum* and *R. padi* have facultative reproduction with both anholocyclic and holocyclic strategies (Poehling et al. 2007). RWA nymph production is temperature-specific, with 32 to 82 nymps per female produced in the 13° - 17°C temperature range (Aalbersberg et al. 1987, Nowierski and Johnson 1995). Variation in greenbug fecundity depends on expression of anholocyclic and/or holocyclic reproduction by different biotypes (Rider and Wilde 1998).

Webster and Starks (1987) compared the fecundity of greenbug biotype E and RWA biotype 1 on TAM 105 wheat at 12-14°C, 19-21°C and 26-28°C. RWA biotype 1 reproduction ranged from 32 - 51 nymphs over a 27 - 29 day period, compared to 74 - 81 greenbug biotype E nymphs produced in only 13-34 days. A classic paper by Dixon (1976) studied reproductive strategies in each morph of the bird cherry-oat aphid, and observed fecundity of 15, 25, and 38 nymphs for gynopara, alate, and emigrants, respectively. This information is very interesting, and points out that variation in populations of other cereal aphids must to be accounted not only for by species morphs of each species. Such information is important in understanding how aphid population fluctuations are related to the time required by each species to colonize and reproduce on different varieties, as well as how these intrinsic aphid factors inteact with climatic conditions at different geographic locations.

The analysis of variance for other cereal aphids (Table 3.2) identified highly significant differences in other cereal aphid populations by location in 2007, 2008, and 2009 (Table 3.3). Mean other cereal aphid populations were high, intermediate, and low in 2007, 2008, and 2009 respectively, at Fort Collins, Colorado compared to Tribune, Kansas and Sidney, Nebraska. At Tribune, low populations occured in 2007, but high populations developed in 2008 and 2009. At Sidney, intermediate populations occured in 2007, but populations were comparatively much lower in 2008 and 2009. Thus, other cereal aphid populations were inconsistent across locations and years. As reported previously in Chapter 2, extremes in climatic conditions limit aphid population establishment and development. In the present study, an additional limitation is the lack of identification of the different aphid species in all other aphid population treatment samples. Based on differences in the other cereal aphid populations by location, differences in population separated by planting date and barley variety were evaluated within each location.

Differences in other cereal aphid populations by planting date and location. Differences in other cereal aphid populations by planting dates were not consistent (Fig. 3.4). Populations in early-planted plots in 2007 were higher than normal plots, but higher populations developed in early-planted plots in 2008 and 2009. These differences could be due to the specific biology and life history of the different aphid species. *R. padi* is a cool season aphid and *S. graminum* is a warm season aphid (Michaud 2010). These differences in life histories for these aphids likely explain some of the inconsistency in the data. A complete identification of the other aphid species samples collected in Colorado, Kansas, and Nebraska during 2007-2009 is needed, in order to assess species specific-interactions by planting date, and to identify other aphid species occurring at particular locations.

Differences in other cereal aphid populations by variety and location. Information regarding the mean other cereal aphid populations by variety at each location for 2007-2009 is presented in Figs. 3.5, 3.6 and 3.7. Fig. 3.5 shows mean other cereal aphid populations for each variety by location in 2007. At Fort Collins and Sidney, Nebraska, Otis_p (under thiamethoxam regime) presented the lowest mean populations, and the two RWA-resistant barley varieties, Sidney and Stoneham, were not significantly different from the susceptible variety Otis. At Tribune, no differences were observed between varieties in mean other cereal aphid populations. Fig. 3.6 shows the interaction of location and variety in 2008. No significant differences were observed between varieties in mean other cereal aphid populations at Fort Collins. At Tribune, the lowest mean aphid populations were observed on Otis_p plants, and no differences in aphid populations were observed between Stoneham, Sydney, or Otis. At Sidney, Nebraska, Stoneham and Sydney plants produced higher populations of other cereal aphids when compared with Otis with- and without thiamethoxam. Fig. 3.7 corresponds to the data obtained for the interaction between location and variety in 2009, and a similar trend was observed at all three locations, with Otis_p yielding the lowest other cereal aphid populations compared to Otis, Stoneham and Sydney. Again, there was no significant difference in aphid populations between Otis, Stoneham and Sydney. In the case of Kansas, Otis presented the highest Mean other cereal aphid populations were highest on Otis at Tribune, and intermediate to populations on Otis at Sidney and Fort Collins.

Differences between varieties were also subjected to orthogonal contrast analysis (Table 3.4). The most important result shown is this table is that with exception of 2008, no differences were observed between Otis and the combined effect of the RWA-resistant varieties, Sidney and Stoneham. Populations on Otis under thiamethoxam protection were significantly different from those on both Otis without seed treatment and the RWA-resistant varieties. These results were similar to those of Macedo et al. (2009), who found that populations of *R. padi* were higher than RWA populations on wheat varieties with RWA antibiosis or tolerance. This also suggests that resistant traits that are effective against one aphid species are not necessarily effective other aphid species.

Differences in other cereal aphid populations by planting date, variety, and location. In 2007, there were highly significant differences in interactions between planting dates and varieties at Fort Collins, Colorado and Sidney, Nebraska, and early planted plots yielded the highest other cereal aphid populations (Table 3.5). Low aphid populations were observed on Otis plants treated with thiamethoxam and no significant differences were observed on Otis plants on different planting dates. At both locations, Sidney and Stoneham plants supported high to intermediate cereal aphid populations that were not significantly different from the means observed in the susceptible variety, Otis. Kansas presented only slight significant differences that are mostly due to planting dates and not due to varieties. Only Otis_p and Stoneham in normal planted plots yielded the lowest cereal aphid mean populations when compared with other varietal treatments.

In 2008 (Table 3.6), early planted plots in Colorado presented the lowest cereal aphids mean populations and no differences were accounted for varietal treatments. In Kansas and Nebraska, no differences were found for planting dates, and statistical differences lay only in varietal treatments, with low levels of cereal aphid mean populations found in Otis_p and high to intermediate levels in populations found in both the susceptible and the RWA-resistant barley varieties.

In 2009 (Table 3.7), normal planted plots in Colorado presented slight increase in cereal aphid populations. The lowest cereal aphid populations in Colorado were recorded in Otis_p in the early planted plot. Only differences by varietal treatment were observed in Kansas 2009, with the highest populations of cereal aphids being recorded in the susceptible variety, intermediate populations in the RWA-resistant varieties, and the lowest populations of cereal aphids in Otis_p. Finally, regarding to Nebraska 2009, early planted plots presented a slight increase in cereal aphids, and Otis_p planted normal had the lowest presence of cereal aphids.

Comparison of RWA and other cereal aphid populations. As shown before, the population of cereal aphids seems to be more affected by varietal treatments than by planting date regimes. In this sense, Figs. 3.8-3.10 show the population levels of RWA vs. other cereal aphids on each varietal treatment by location, during 2007, 2008, and 2009 respectively.

The two RWA-resistant barley varieties, Sidney and Stoneham, yielded low to intermediate levels of RWA when compared to the susceptible variety Otis. Also, the levels of infestation in Sidney and Stoneham were as low as the ones observed in the seed treatment, Otis_p. In contrast, other cereal aphid populations were similar in the two RWA-resistant barley varieties when compared to the susceptible barley variety, Otis, in Colorado and Nebraska (2007); and in the Kansas and Nebraska (2008) sites. No differences between varietal treatments for other cereal aphid populations were seen in Kansas (2007) and Colorado (2008). In the specific case of Nebraska (2008), the two RWA-resistant barley varieties presented higher cereal aphid populations than Otis. A general trend that can be seen in the Figs.3.8-3.10 is that Sidney and Stoneham RWA-resistant varieties seem to be highly preferred by other cereal aphids, regardless the level of resistances against RWA. This result is also in accordance with the orthogonal contrasts previously shown. Results found by Messina and Bloxham (2004) support these findings. They showed that the performance of RWA was reduced in the RWA-resistant wheat lines, but these resistant lines did not affect population growth of *R. padi*. Also, these authors suggested that an induce resistance response rather than a constitutively resistance was responsible for the differences in the aphid performance.

To summarize, two general trends were observed in this research. First, the RWA-resistant barley varieties yielded lower RWA populations when compared to the susceptible variety, Otis. However, this is not true for other cereal aphids, which had higher population levels in the Sidney and Stoneham varieties when compared to Otis. Second, Otis under thiamethoxam regime (Otis_p) seems to reduce the cereal aphid mean populations in a more effective way than RWA populations. Qureshi and Michaud (2005b) showed that in some cases there are alterations in plant physiology that are induced by aphid feeding, and that some cases can have positive, neutral, or negative impacts in other aphid species that occur in the same plants. In this sense, I suggest the need to develop further studies in order to determine the effect on other cereal aphids when feeding on barley with tolerance characteristics that are specific to RWA. Michaud and Sloderbeck (2005) stated that seed treatment provided early season protection to crops being attack by RWA populations. The seed treatment with neonicotinoids such as thiamethoxam has been extensively used due to an effective action on insect nicotinic acetylcholine receptors (nAChR) with low residual effects on crops and because this chemical treatment offers control to hemipteran insect pests such as aphids, whiteflies, and planthoppers (Nauen et al. 2003). However, insecticide resistance has been previously recorded for several aphid species (e.g. *Myzus persicae*, *Aphis gossypii*, and *S. graminum* in the U.S.) (Foster et al. 2007), and even though insecticide resistance has not been previously recorded in RWA biotype populations, it would be fair to assume that the high variation in RWA populations in the seed protection regime (Otis_p) could be due to the presence of diversity of RWA populations or biotypes across the experimental areas (Burd et al. 2006, Weiland et al. 2008).

Conclusions

In general, populations of cereal aphids other than RWA were higher at Tribune, Kansas and Fort Collins, Colorado than at Sidney, Nebraska. However, the validity of these differences should be further assessed by contrasting the populations of specific aphid species with climatic conditions at each of the three sites in order to establish trends between aphid population dynamics and climatic requirements. Identification of cereal aphid species are also required in order assess the aphid species - planting date interactions, No clear responses of other cereal aphid species to altered planting dates were observed, . The presence of RWA-resistant varieties in eastern Colorado, western Kansas and western Nebraska did not reduce other cereal aphid populations. On the contrary, the RWA-resistant varieties Sidney and Stoneham were highly preferred by other cereal aphids regardless of the level of RWA resistance, as there were no differences in other aphid populations between Sidney, Stoneham and the RWA-susceptible variety Otis. Significantly higher populations occurred on plants of Sidney, Stoneham and Otis than on Otis plants protected with thiamethoxam insecticide, indicating that seed treatment with thiamethoxam is effective in reducing both RWA and other cereal aphid populations. However, in order to avoid insecticide resistance, continuous monitoring of the target aphid pest and a rational use of seed insecticide is highly desirable.

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Tables and Figures

Table 3.1 Cereal crop production rank, area harvested, and yield in the United States in 2008 and 2009.

Cereal crop	World rank		Area harvested (Ha)		Yield (Hg/Ha)	
	2008	2009	2008	2009	2008	2009
Barley	2	5	1,529,324	1,259,800	34,176	39,286
Buckwheat	7	5	83,000	83,000	10,000	10,000
Maize	1	1	31,796,493	32,209,277	96,583	103,389
Millet	-	-	186,157	118,574	18,127	18,868
Oats	-	-	566,566	558,068	24,112	24,209
Rice	11	12	1,204,357	1,255,753	76,716	79,412
Rye	-	-	108,862	101,982	18,618	17,417
Sorghum	1	3	2,942,500	2,233,890	40,779	43,548
Wheat	3	3	22,540,828	20,181,081	30,177	29,886

Ha: Hectare; Hg/Ha: Hectogram per hectare

Source: FAO Statistics, 2010

Table 3.2 Analysis of variance for other cereal aphid populations collected at sites at Fort Collins, Colorado; Tribune, Kansas and Sidney Nebraska in 2007, 2008, and 2009.

Effect	Loc	DF	2007		2008		2009	
			F value	Pr > F	F value	Pr > F	F value	Pr > F
Loc		2	7.66	0.0064	12.72	0.0024	75.63	<.0001
Pltdate	Across locations	1	30.05	<.0001	17.95	0.0004	0.01	0.9207
Loc*Pltdate	Across locations	2	0.29	0.7535	5.01	0.0166	5.12	0.0129
Loc*Pltdate	CO	1	7.47	0.0125	25.30	<.0001	5.97	0.0213
Loc*Pltdate	KS	1	8.99	0.0069	1.20	0.2865	0.04	0.8351
Loc*Pltdate	NE	1	14.17	0.0011	1.47	0.2381	4.24	0.0491
Variety	Across locations	3	14.63	<.0001	6.32	0.0005	25.04	<.0001
Loc*Variety	Across locations	6	3.28	0.0119	1.52	0.1776	5.18	0.0004
Loc*Variety	CO	3	9.41	0.0001	0.08	0.9686	2.68	0.0588
Loc*Variety	KS	3	0.49	0.6923	5.59	0.0012	28.36	<.0001
Loc*Variety	NE	3	11.29	<.0001	3.68	0.0140	4.37	0.0090
Pltdate*variety	Across locations	3	3.85	0.0135	1.14	0.3348	2.53	0.0673
Loc*Pltdate*Variety	Across locations	6	1.33	0.2572	2.06	0.0620	1.85	0.1074
Loc*Pltdate*Variety	CO	7	5.85	<.0001	4.69	0.0002	2.11	0.0597
Loc*Pltdate*Variety	KS	7	2.04	0.0683	3.32	0.0033	12.81	<.0001
Loc*Pltdate*Variety	NE	7	8.36	<.0001	2.25	0.0364	4.38	0.0008

Analysis of variance determined with Proc MIXED, with fixed effects SE method, and degrees of freedom obtained by Satterthwaite method (See Appendix B for SAS Codes).

Total number of observations for the analysis= 192

Number of observations per location= 64, observations per planting date= 96, observations per variety= 48

Number of observations per planting date/location = 32

Number of observations per variety/location = 16

Number of observations per variety/planting date/location = 8

Table 3.3 Mean ± S. E. other cereal aphid populations across planting dates and varieties collected at sites at Fort Collins Colorado;Tribune, Kansas and Sidney Nebraska in 2007, 2008 and 2009.

Location	**Mean ± S. E. other cereal aphids**					
	2007[a]		**2008**		**2009**	
Colorado	8.4 ± 0.973	a	10.7 ± 2.044	b	6.2 ± 0.465	b
Kansas	3.7 ± 0.629	b	20.9 ± 2.632	a	71.2 ± 7.458	a
Nebraska	6.5 ± 1.209	ab	2.69 ± 0.817	c	9.9 ± 0.986	b

[a] Means followed by the same letter for each year are not significantly different (pr<|t| = 0.05)

Table 3.4 Orthogonal contrasts obtained in order to determine varietal differences in mean cereal aphid populations in 2007, 2008 and 2009 at Fort Collins, Colorado; Tribune, Kansas and Sidney, Nebraska.

Contrast	DF	2007		2008		2009	
		F	Pr > F	F	Pr > F	F	Pr > F
Otis versus Otis_p	1	32.12	<.0001**	3.83	0.0526 *	60.18	<.0001 **
Otis versus Resistant varieties	1	0.25	0.6204 n. s.	4.12	0.0445*	2.81	0.1009 n. s.
Otis_p vs. Resistant varieties	1	36.54	<.0001**	18.39	<.0001**	53.01	<.0001**

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam regime, Resistant varieties = Combined effect of Sidney and Stoneham varieties.

Orthogonal contrast determined with PROC MIXED (SAS 2003)

** Significant at 1%,* significant at 5%, n. s. = non significant differences

Table 3.5 Other cereal aphid mean populations by location, planting date and variety interactions in 2007

Location	Planting date	Variety	Mean ± S. E.	
Colorado**	Early	Otis	15.3 ± 3.524	a
		Otis_p	3.5 ± 2.816	c
		Sidney	12.9 ± 2.030	a
		Stoneham	12.4 ± 2.427	a
	Normal	Otis	8.8 ± 2.396	ab
		Otis_p	1.8 ± 0.491	c
		Sidney	6.5 ± 2.428	ab
		Stoneham	6.3 ± 1.790	ab
Kansas *	Early	Otis	4.1 ± 1.856	a
		Otis_p	4.0 ± 1.282	a
		Sidney	6.0 ± 2.712	a
		Stoneham	5.3 ± 2.562	a
	Normal	Otis	4.9 ± 1.767	a
		Otis_p	1.8 ± 0.840	b
		Sidney	2.0 ± 0.866	ab
		Stoneham	1.9 ± 1.469	b
Nebraska **	Early	Otis	9.3 ± 2.908	ab
		Otis_p	0.8 ± 0.412	cd
		Sidney	19.5 ± 6.361	a
		Stoneham	9.5 ± 3.417	ab
	Normal	Otis	4.0 ± 0.802	b
		Otis_p	0.5 ± 0.267	d
		Sidney	3.8 ± 1.953	c
		Stoneham	5.0 ± 1.102	b

Means followed by the same letter for each location are not significantly different (pr<|t| = 0.05)

** Significant at 1%,* significant at 5%, n. s. = non significant differences

Table 3.6 Other cereal aphid mean populations by location, planting date, variety interaction in 2008

Location	Planting date	Variety	Mean ± S. E.	
Colorado **	Early	Otis	2.0 ± 1.376	b
		Otis_p	2.6 ± 1.475	b
		Sidney	5.5 ± 2.549	b
		Stoneham	8.9 ± 4.525	ab
	Normal	Otis	19.4 ± 5.227	a
		Otis_p	17.4 ± 5.538	a
		Sidney	12.8 ± 3.478	a
		Stoneham	17.4 ± 12.288	ab
Kansas **	Early	Otis	17.4 ± 3.354	ab
		Otis_p	5.4 ± 1.100	c
		Sidney	14.6 ± 3.664	bc
		Stoneham	23.6 ± 7.729	b
	Normal	Otis	19.4 ± 9.151	ab
		Otis_p	14.0 ± 5.538	bc
		Sidney	39.3 ± 10.097	a
		Stoneham	34.0 ± 8.600	a
Nebraska *	Early	Otis	0.6 ± 0.375	c
		Otis_p	0.1 ± 0.125	c
		Sidney	1.9 ± 1.186	abc
		Stoneham	7.5 ± 5.490	ab
	Normal	Otis	1.9 ± 0.854	ab
		Otis_p	0.8 ± 0.490	c
		Sidney	6.1 ± 2.467	a
		Stoneham	2.6 ± 1.413	ab

Means followed by the same letter for each location are not significantly different (pr<|t| = 0.05)

** Significant at 1%,* significant at 5%, n. s. = non significant differences

Table 3.7 Other cereal aphid mean populations by location, planting date, variety interaction in 2009

Location	Planting date	Variety	Mean ± S. E.	
Colorado *	Early	Otis	6.1 ± 1.684	ab
		Otis_p	2.1 ± 0.667	b
		Sidney	3.9 ± 0.742	b
		Stoneham	6.9 ± 1.846	a
	Normal	Otis	9.0 ± 2.155	a
		Otis_p	6.0 ± 1.773	ab
		Sidney	7.5 ± 1.323	a
		Stoneham	8.1 ± 1.231	a
Kansas **	Early	Otis	129.9 ± 21.038	a
		Otis_p	16.9 ± 4.164	c
		Sidney	50.1 ± 9.234	b
		Stoneham	68.3 ± 15.220	ab
	Normal	Otis	156.0 ± 48.379	a
		Otis_p	9.5 ± 2.770	c
		Sidney	72.8 ± 11.250	ab
		Stoneham	65.9 ± 14.197	ab
Nebraska **	Early	Otis	6.9 ± 2.013	b
		Otis_p	9.6 ± 3.635	b
		Sidney	10.6 ± 3.401	b
		Stoneham	20.9 ± 4.458	a
	Normal	Otis	13.5 ± 2.866	ab
		Otis_p	2.0 ± 0.597	c
		Sidney	6.5 ± 2.146	b
		Stoneham	9.6 ± 3.479	b

Means followed by the same letter for each location are not significantly different (pr<|t| = 0.05)

** Significant at 1%,* significant at 5%, n. s. = non significant differences

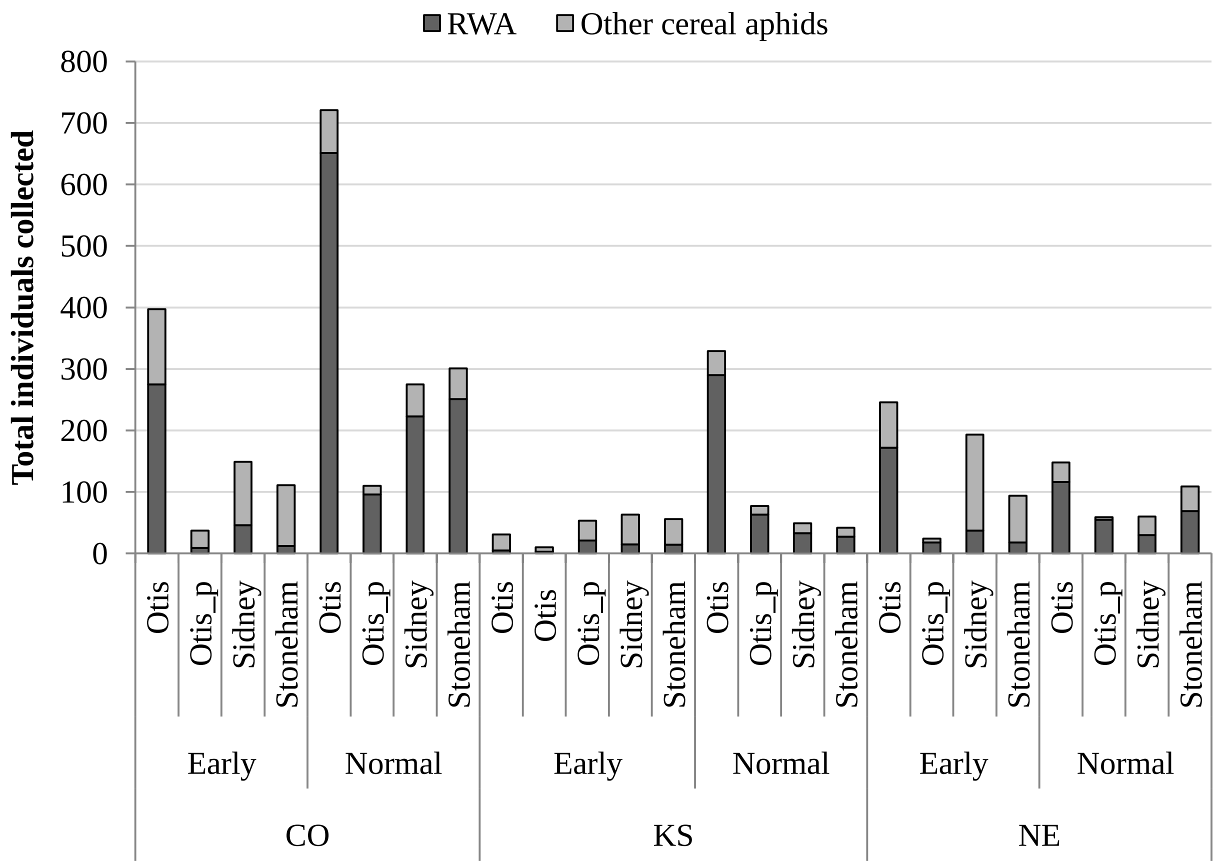

Fig. 3.1 Abundance of RWA and other cereal aphids by location, planting date and variety in 2007.

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam regime, Resistant varieties = Sidney and Stoneham.

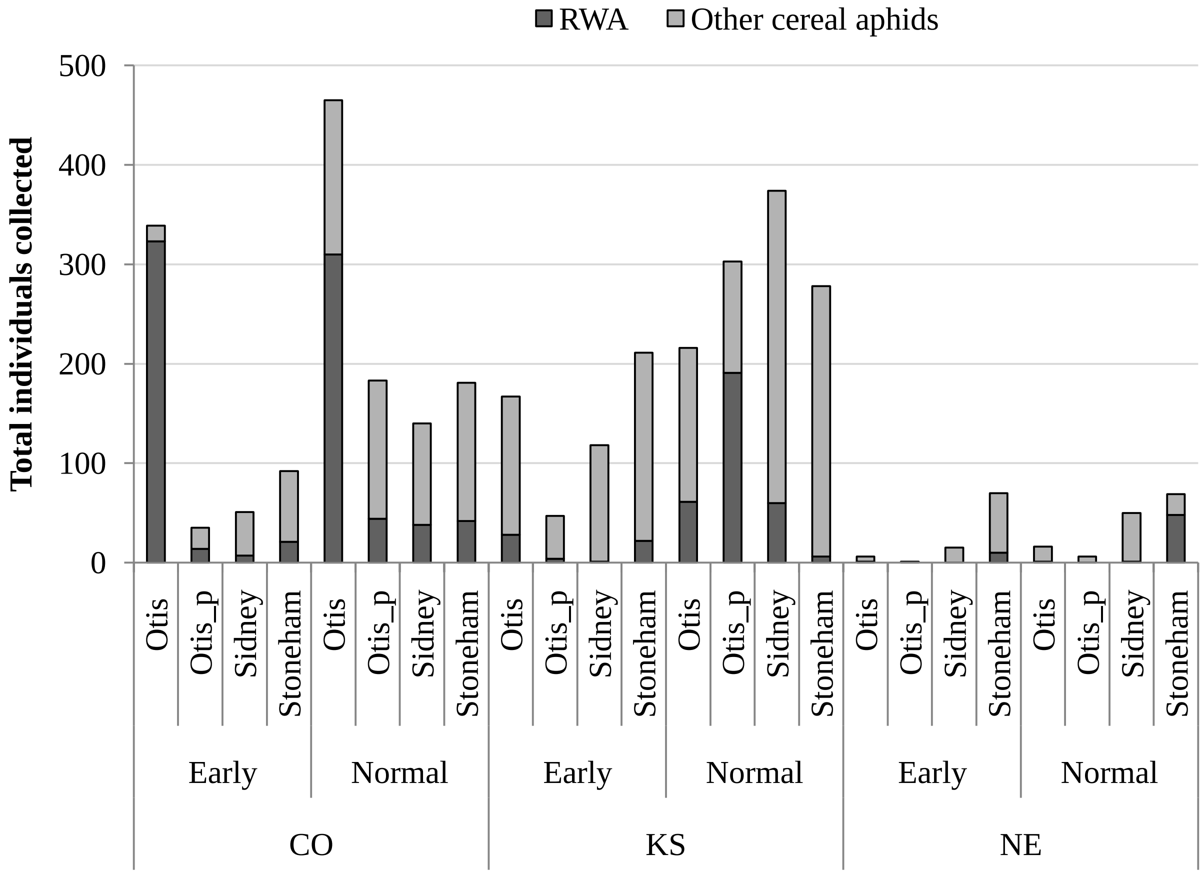

Fig. 3.2 Abundance of RWA and other cereal aphids by location, planting date and variety in 2008.

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam regime, Resistant varieties = Sidney and Stoneham.

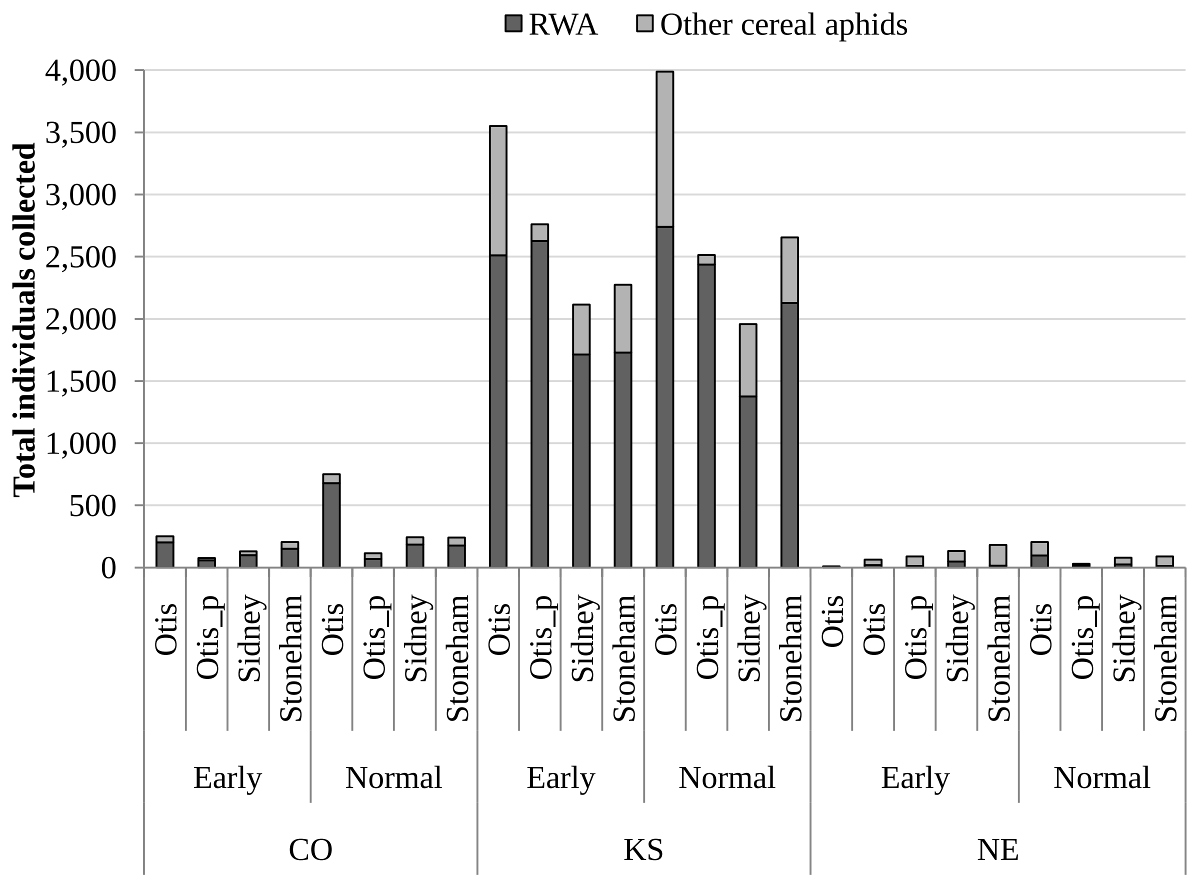

Fig. 3.3 Abundance of RWA and other cereal aphids by location, planting date and variety in 2009.

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam regime, Resistant varieties = Sidney and Stoneham.

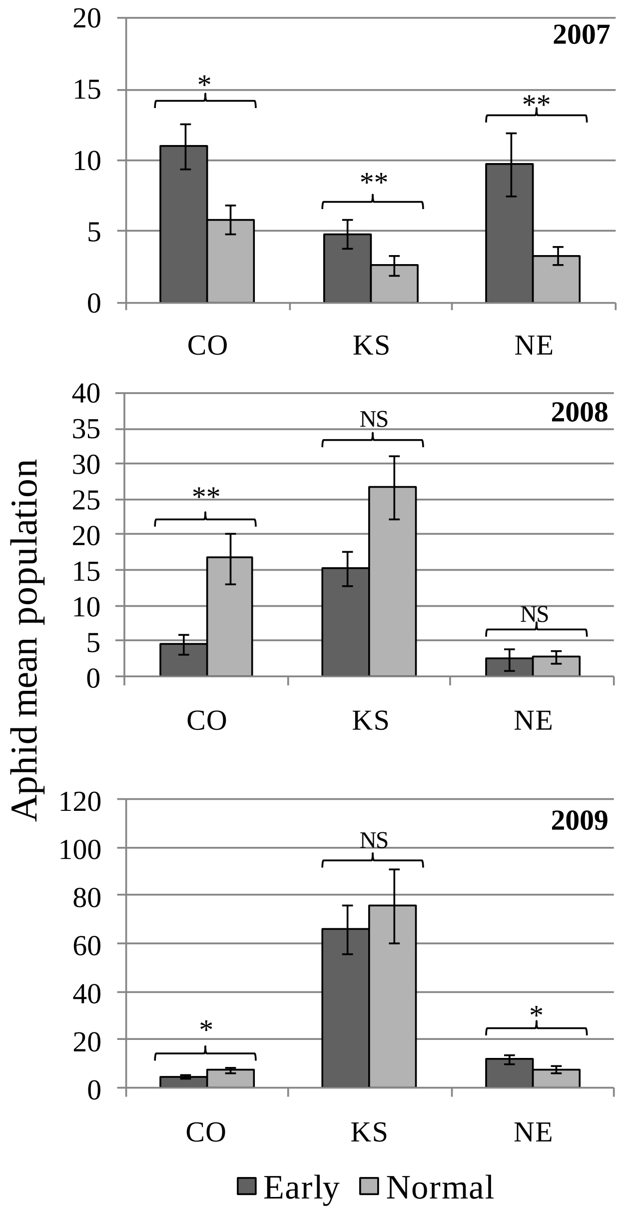

Fig. 3.4 Mean other cereal aphid populations present on barley plants in early planted plots and plots planted on normal planting dates at Fort Collins, Colorado; Tribune, Kansas and Sidney, Nebraska in 2007, 2008, and 2009.

Significant differences were obtained for planting dates within each location for each year.

** Significant at 1%, * significant at 5%, n. s.= non significant difference

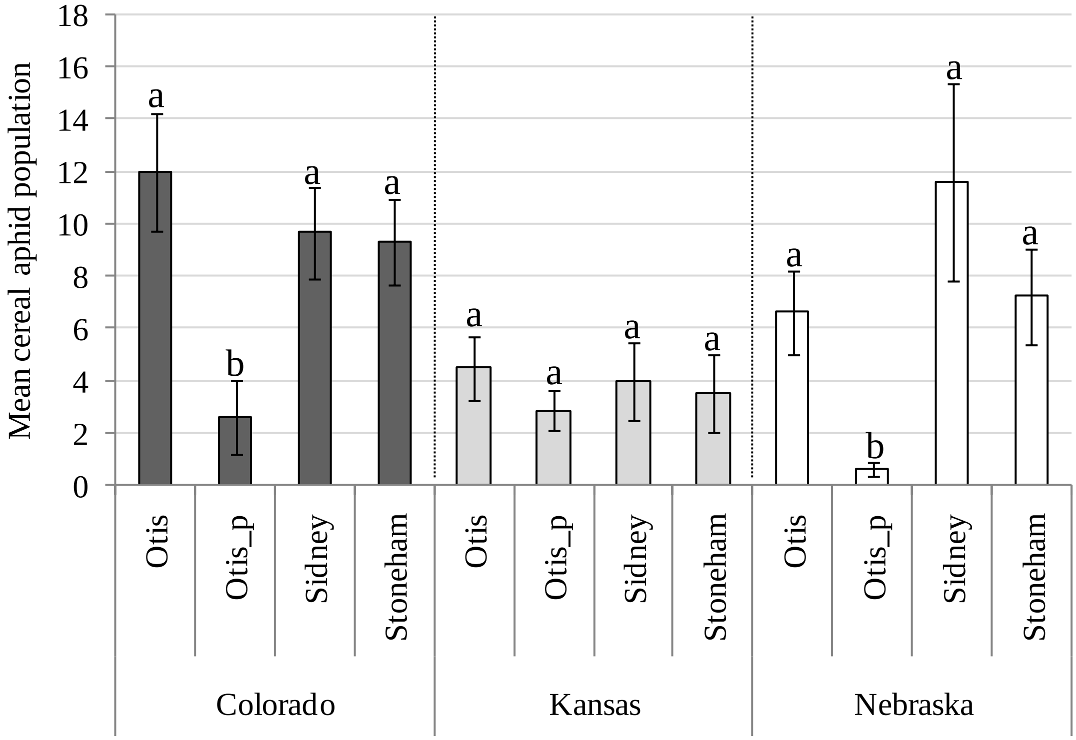

Fig. 3.5 Mean other cereal aphid populations present on plants of Stoneham, Sidney and Otis barley, and Otis barley treated with thiametoxam averaged across plaing date at Fort Collins, Colorado; Tribune, Kansas and Sidney, Nebraska in 2007.

Means followed by the same letter for each location are not significantly different (pr<|t| = 0.05).

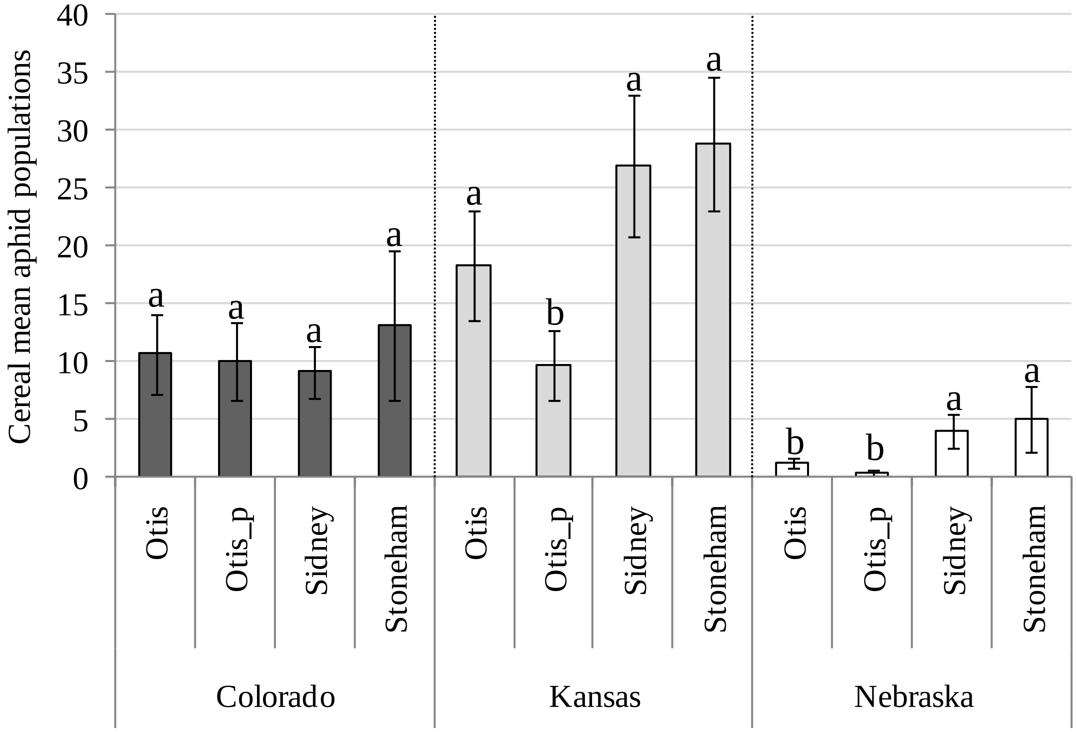

Fig. 3.6 Mean other cereal aphid populations present on plants of Stoneham, Sidney and Otis barley, and Otis barley treated with thiametoxam averaged across plaing date at Fort Collins, Colorado; Tribune, Kansas and Sidney, Nebraska in 2008.

Means followed by the same letter for each location are not significantly different (pr<|t| = 0.05).

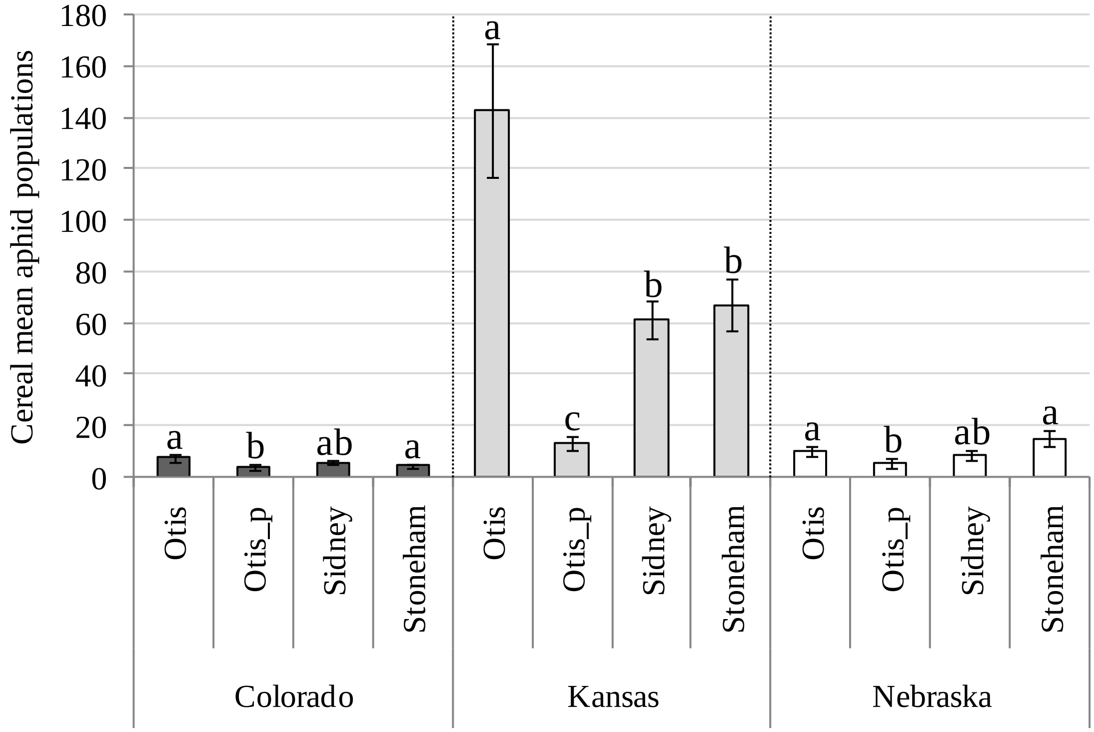

Fig. 3.7 Mean other cereal aphid populations present on plants of Stoneham, Sidney and Otis barley, and Otis barley treated with thiametoxam averaged across plaing date at Fort Collins, Colorado; Tribune, Kansas and Sidney, Nebraska in 2009.

Means followed by the same letter for each location are not significantly different (pr<|t| = 0.05).

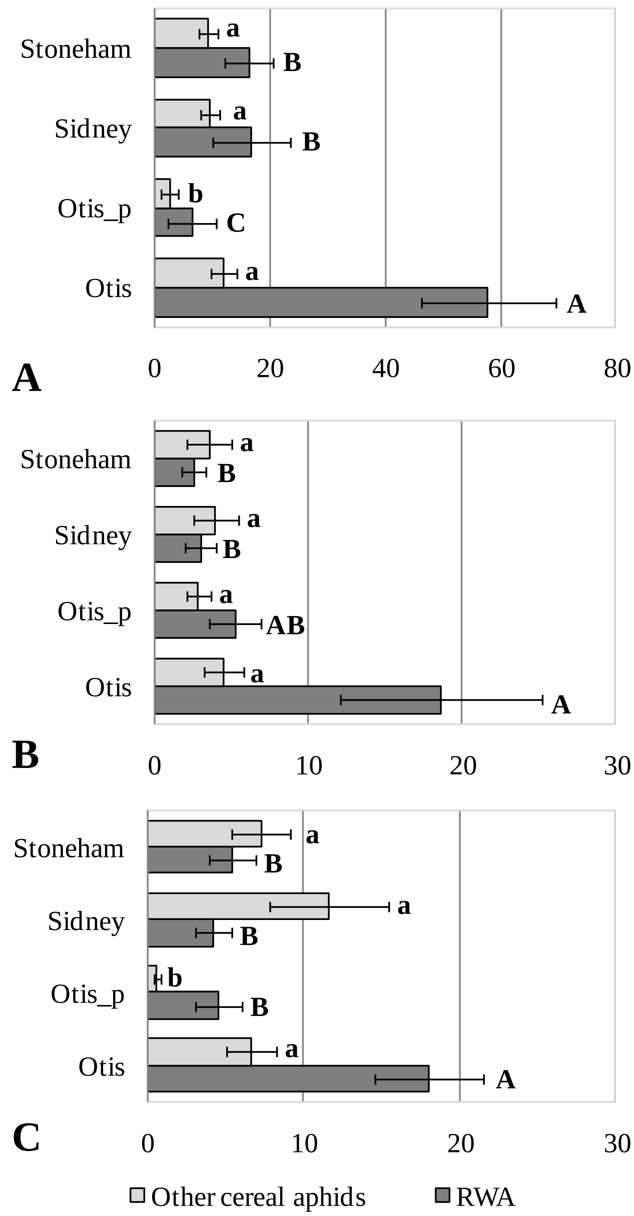

Fig. 3.8 Comparison of mean RWA and other cereal aphid populations by variety at A) Fort Collins, Colorado; B) Tribune, Kansas; and C) Sidney, Nebraska in 2007.

Means followed by the same letter for each location are not significantly different (pr<|t| = 0.05). Capital letters = Differences in mean RWA populations, Lower case letters = differences in mean other cereal aphids populations.

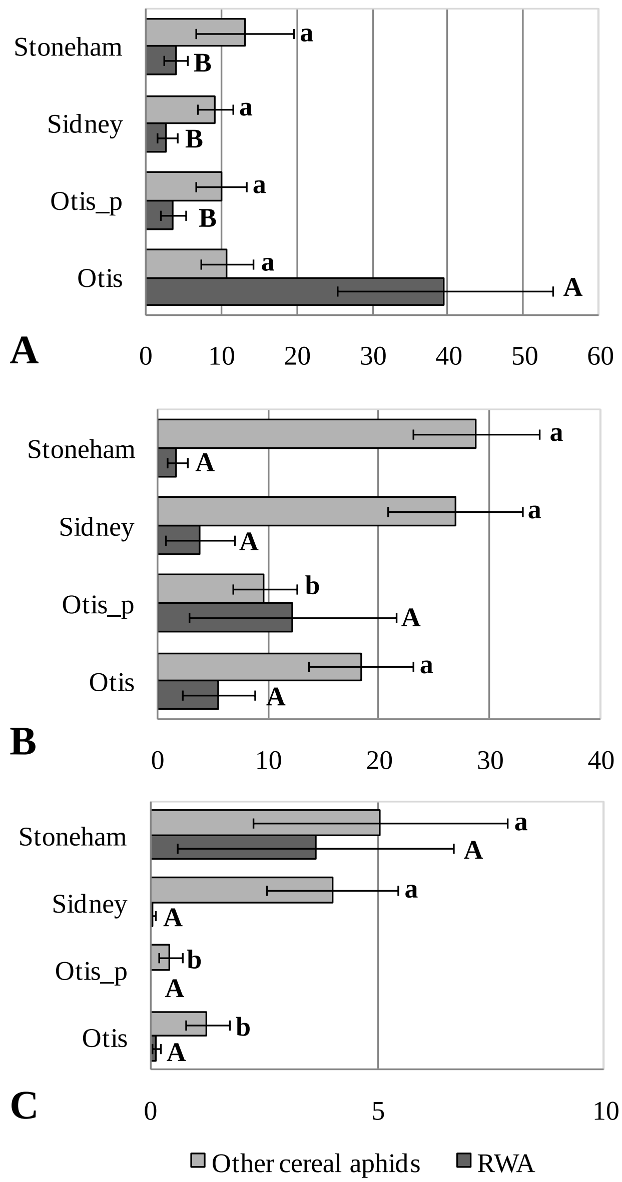

Fig. 3.9 Comparison of mean RWA and other cereal aphid populations by variety at A) Fort Collins, Colorado; B) Tribune, Kansas; and C) Sidney, Nebraska in 2008.

Means followed by the same letter for each location are not significantly different (pr<|t| = 0.05).

Capital letters = Differences in mean RWA populations, Lower case letters = differences in mean other cereal aphids populations.

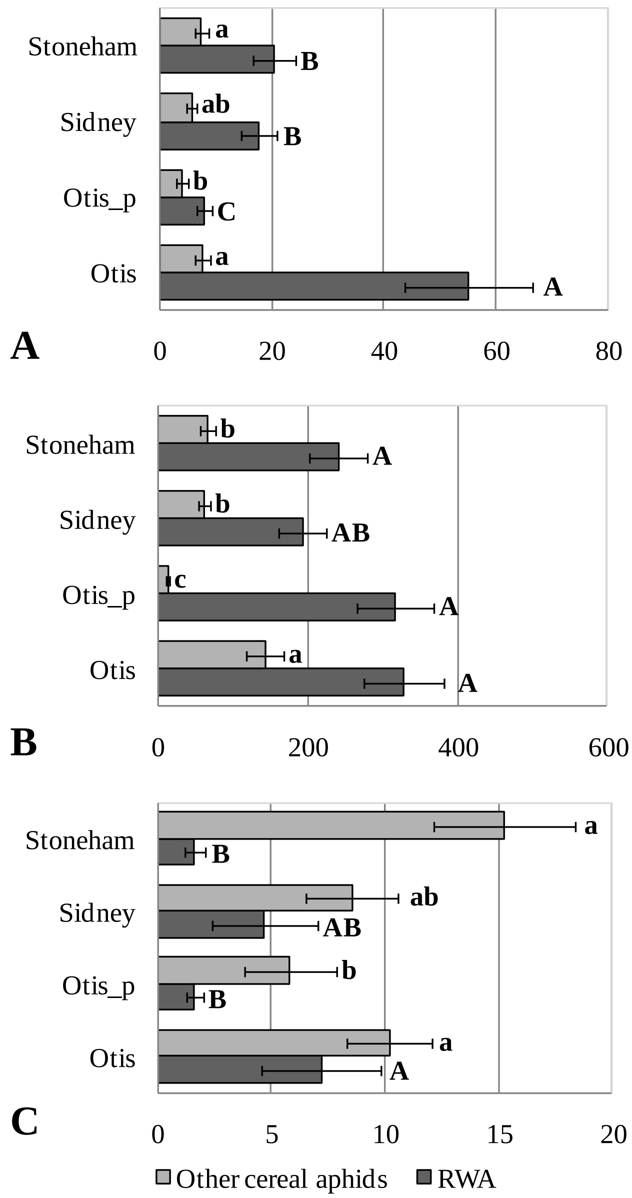

Fig. 3.10 Comparison of mean RWA and other cereal aphid populations by variety at A) Fort Collins, Colorado; B) Tribune, Kansas; and C) Sidney, Nebraska in 2009.

Means followed by the same letter for each location are not significantly different (pr<|t| = 0.05).

Capital letters = Differences in mean RWA populations, Lower case letters = differences in mean other cereal aphids populations.

CHAPTER 4 - EFFECT OF CULTURAL PRACTICES AND RWA-RESISTANT BARLEY VARIETIES ON THE NATURAL OCCURRENCE OF RWA BIOLOGICAL CONTROL AGENTS IN COLORADO, KANSAS, AND NEBRASKA

Abstract

Cereal crops are attacked by several aphid species across the cereal growing regions of the world, with Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (RWA), being one of the most economically important introduced pests in the United States. In order to control this pest, an RWA integrated pest management should attempt to include several practices, such as altered planting date, insecticide seed treatment, aphid-resistant varieties and conserve and enhance populations of exotic and native natural enemies. However, in the particular case of barley there is little or no information about the effect(s) of the above-mentioned pest management tactics on the natural occurrence of natural enemy populations in the field. For that reason, the objective of this research was to understand how the use of integrated pest management (IPM) practices against RWA affects the presence of natural enemies in eastern Colorado, western Kansas and western Nebraska. Planting dates and RWA-resistant varieties were evaluated in a split-plot design with two main plot treatments (early and normal planting dates) in experimental fields at Fort Collins, Colorado; Tribune, Kansas and Sidney, Nebraska during 2007, 2008 and 2009. Four split plot variety treatments (RWA-resistant Stoneham and Sydney, RWA-susceptible Otis, and Otis treated with thiamethoxam) were randomized within each main treatment plot. Natural enemy abundance was similar on plants of all varieties, including Otis plants treated with thiamethoxam. Thus, systemic, short residual barley seed – protection from thiamethoxam had no adverse affect on cereal aphid natural enemies. Natural enemies were encountered with more frequency on plants from plots planted at normal planting times (mid April) but no definitive effect of planting date was determined.

Key words: Parasitoids, predators, cereal aphids, abundance index, tolerant varieties

Introduction

Cereal crops are attacked by several aphid species across the cereal- growing regions of the world. One of the most important examples is the spread of Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (RWA) from crescent region in the Middle East to new regions in South Africa, North, Central and South America in the last 30 years. Since its initial accidental introduction, RWA has become a limiting factor in barley and wheat production in the U.S. Western Plains, causing hundreds of millions of dollars of losses through reduced yields and increased insecticide treatment costs (Webster et al. 2000, Michaud and Sloderbeck 2005, Gutshe et al. 2009).

One of the major strategies to control aphids and other insect pest worldwide was with no doubt the extensive use of pesticides products n 1950s and 1960s. However, after several years, the agricultural community realized the environmental problems related with this chemical products. In that sense, other management strategies were developed and they were defined as integrated management strategies that limit the use of chemical control and enhance the use of alternative strategies such as cultural control, host plant resistance, and biological control (Stern et al. 1959, Miller and Pike 2002, Pedigo and Rice 2006)

One of the major limitations in the control of RWA populations in the United States was the lack of biological control agents for this introduced pest and the use of cereal crops that were susceptible to the attack of the aphid. For that reason, the efforts have been focused on the development of resistant varieties and to increase the populations of exotic and native natural enemies. This combined effect can help to reduce the economic impact of RWA (Holtzer et al. 1996, Michaud and Sloderbeck 2005).

Efforts to develop resistant varieties increase from 1990 to 1993, where 24,000 barley accessions were evaluated for resistance by USDA-ARS researchers at Stillwater, OK. Of these, 109 accessions presented some level of resistance and the main category of resistance expressed is tolerance (Mornhinweg et al. 2006). Two of these accessions were advanced to produce the RWA-resistant lines STARS-9301B and STARS-9577B (Mornhinweg et al. 1995, 1999). One particular example is the development of two RWA-resistant barley varieties, Sidney and

Stoneham that were obtained from crossesbetween 'Otis', a commercial but highly RWA susceptible variety, and STARS 9301B and STARS 9577B, respectively(Bregitzer et al. 2008, Mittal et al. 2009).

In biological control approach, the importance increased greatly in the late 1980's when several hundred species of predators and parasitoids were released in 18 U.S. states and Canada from 1987 to 1997 after the USDA 'National RWA Integrated Pest Management Program' was initiated (Mohamed et al. 2000, Brewer and Elliot 2004, Lee et al. 2005, Powell and Pell 2007). The objective of this program was to import aphid natural enemies from the native origin of RWA in Eurasia, mostly from Morocco and Middle East (Tanigoshi et al. 1995). In one decade, more than 11.8 million individuals of 11 parasitoids species, were release. As stated by Michaud and Sloderbeck (2005), this big biological control effort grouped more than 120 scientists from 20 countries which imported beneficial insects of at least 24 species in 16 affected states in the United States. Currently, some of the most important biological control agents and more used are braconid parasitoids (*Aphidius*, *Lysiphlebus*, and *Aphelinus*), ladybird beetles (*Adalia*, *Coleomegilla*, *Harmonia*, *Hippodamia*), lacewing (Neuroptera: Chrysopidae, Hemerobiidae), predatory bugs (Hemiptera: Anthocoridae, Geocoridae, and Nabidae), and fungal pathogens (*Beauveria bassiana*, *Paecilomyces fumosoroseus*, *Lecanicillium* spp.) (Elliot et al. 1998, Michels et al. 2001, Powell and Pell 2007).

In addition to classical biological control, efforts have involved conservation by manipulating and enhancing the role of the natural enemies (Powell and Pell 2007). One of the most used control strategies on cereals like wheat and barley has been chemical protection with systemic insecticides like neonicotinoids because they offer good protection against RWA and other cereal aphids when applied as seed treatments (Dewar 2007). In addition, it is well known that cultural practices such as altered sowing date may affect the rate of growth, reproduction, and dispersal not only of the target pest but also of their natural enemies (Wratten et al. 2007). In the case of aphids, early planting can affect aphid colonization of the crop, therefore reducing the stress inflicted in the plants, even in those tolerant to attack (Peairs 1998). Finally, the use of resistant barley and other cereal crops can contribute reduced aphid populations. However, there is insufficient information about the effect of the above-mentioned pest management strategies on the occurrence of natural enemy populations in barley production. For that reason, the objective of this research is to understand how the use of integrated pest management (IPM) practices against RWA affects the incidence of natural enemies in eastern Colorado, western Kansas and western Nebraska.

Materials and Methods

Location

The present study was conducted at three locations within the Great Plains biogeographic region during 2007, 2008, and 2009. These study sites were located at the University of Nebraska High Plains Agricultural Laboratory near Sidney, Nebraska (41^{0}12’21.91’’ N, 103^{0}00’42.72” W, elevation: 1320 m), the Colorado State University Agricultural Research, Development and Education Center (ARDEC), near Fort Collins, Colorado (40^{0}39’09.37” N, 105^{0}00’01.67” W, elevation: 1550 m), and the Kansas State University Research farm near Tribune, Kansas (38^{0}27.56.03” N, 101^{0}45’40.17” W, elevation: 1100 m).

Experimental Design and Treatments

The experimental design of the trials was a split plot design with two main plot treatments (planting dates) replicated eight times in a randomized complete block design. The early planted plots were sown on March 21-23, 2007, March 11-13, 2008, and March 15-19, 2009. Plants in normal planted plots were sown on April 22, 2007, April 12-13, 2008, and April 13 and 19, 2009. Within each planting date, four split-plot treatments (varieties) were randomized. These treatments included the RWA-resistant barley cultivars Stoneham and Sidney, and the susceptible cultivar Otis under thiamethoxam-protected and unprotected regimes (Otis_p and Otis, respectively). Individual subplots were 9.15 m wide and 12.2 m long. Alleys were treated with glyphosate to eliminate plants harboring background aphid populations developing in these areas. Individual sub-plots were 9.15 m wide and 12.2 m long. Alleys were treated with glyphosate to eliminate plants harboring background aphid populations developing in these areas. The pedigree of Sidney barley is (Otis x STARS 9301B) and the pedigree of Stoneham is (Otis x 9577B). Resistance in Stoneham is linked to two quantitative trait loci (QTLs), one in the short arm of chromosome 1H and one in the long arm of chromosome 3H. Resistance in Sidney is linked to the two QTLs associated with Stoneham and a different QTL on chromosome

2H (Nieto-Lopez and Blake 1994, Mornhinweg et al. 2006, Bregitzer et al. 2008, Mittal et al. 2008, Mittal et al. 2009).

Natural Enemy Sampling

During the growing season, predators and parasitoid wasps were collected during the barley tillering stage, jointing stage, boot stage, and early dough stage. In the early-planted plots, these stages occurred at approximately early May, mid May, early June and late June, respectively, and in the normal-planted plots, these stages occurred at approximately mid-May, early June, late June, and early July. To obtain natural enemies, a D-vac sampler (STIHL SH 55/85) (STIHL Inc., Virginia Beach, Va) was run in two randomly-selected 1-meter length sections of each barley row per split-plot treatment. The D-vac machine employs a suction technique and has been used previously to collect natural enemies (Elliot et al 2006). For each variety within each planting date, the two samples were pooled. The specimens within the pooled samples were rinsed with water to remove dust and then preserved in 80% ethyl alcohol until they could be inspected. Specimens were later examined in the laboratory using a stereomicroscope, as needed, to assist with identifications. Specimens were sorted and the numbers in each of the following broad taxonomic groups were recorded: spiders, parasitic wasps, coccinellid beetles, predaceous Hemiptera (Anthocoridae, Geocoridae, Nabidae, Pentatomidae) and lacewings (Neuroptera: Crysophidae and Hemerobiidae). Parasitic wasps were classified by families and some specimens were idenfied to genera. However, due to the low representation in several groups, they were counted and analyzed as a whole group.

Data Analysis

In order to evaluate the effects of planting date and barley variety on natural enemies, data was analyzed using SAS PROC MIXED procedure (SAS 2003), which accounts for random effects, blocks and their interactions within locations, planting dates and varieties. The analysis of variance evaluated individual effects such as location, planting date and variety separately, and the interaction of more than one individual effect. Differences between mean treatments were determined using a t-test for pairwise comparisons with $pr<|t| = 0.05$.

In order to compare diversity between varieties, Shannon-Wiener indices of natural enemy diversity and abundance were evaluated by calculating the proportional abundance or structure of natural enemies present on plants of each variety (Magurran 1988, Jayaraman 2000, Moreno 2001). The Shannon-Wiener index, H', is defined as the ratio of the number of species to their importance within a trophic level or community (Moreno 2001). In this case, each variety was accounted as a different community.

For each location, the total number of natural enemies in each taxonomic group per subplot (varietal treatment) within each planting date was obtained by totaling the counts across the four sample dates. Mean numbers were obtained from the number of natural enemies in each of the eight replications per variety and per planting date. Data were transformed (Log [number of aphids + 1]) to reduce dispersion and attain normality. The relative abundances of natural enemies were computed by showing the proportion of the total number of natural enemies represented by each natural enemy group. As stated by Magurran (1988), the Shannon-Wiener index value, H', expresses the degree of uniformity across all species (S) in the sample. It also measures the average degree of uncertainty in predicting which species belong to an individual chosen at random from a collection. This index takes values between zero and logarithm of S. The value is zero when there is only a single species, meaning that there is no uncertainty about predicting the species from an individual chosen at random. The extreme value is the logarithm of S, when all species are represented by the same number of individuals (Magurran 1988; Moreno 2001).

$$H' = - \Sigma p_i \ln p_i$$

The null hypothesis in this research is that the diversity of the samples coming from the four varietal treatments are equal or, in other words, the RWA tolerance in the resistant barley varieties is not affecting the abundance and diversity of natural enemies. In order to test the null hypothesis, Moreno (2001) recommends following the procedure proposed by Hutcheson (1970). First, calculate the weighted index of diversity (H_w) depending on the frequency of each species. In this case, the H_w is calculated for each varietal treatment.

$$H_w = \frac{(N \log N)\ (\Sigma n_i \log n_i)}{N}$$

Where, n_i= Frequency (number of individual) in each family, species or subdivision.

Second, it was necessary to determine the variance for each weighted index (V_{Hw}) of diversity using the following formula:

$$VHw = \frac{[(\Sigma n_i \log^2 n_i) - (\Sigma n_i \log n_i)^2]/N}{N^2}$$

Where N= total individuals collected in each varietal treatment.

Third, it was necessary to test the null hypothesis using a t-test. In this particular case the t-test is assess using pair-wise comparison between two varietal treatments. Before the calculation of the t-test it was necessary to find the difference of variance between any two varietal treatments was calculated (Dvar).

$$Dvar = \sqrt{Var\ 1 + Var\ 2}$$

Fourth, the t-statistic (t) was calculated for each pair-wise comparison between varietal treatments.

$$t = \frac{Hw_1 - Hw_2}{Dvar}$$

Fifth, the degree of freedoms (df) associated with the value of the t-statistic were calculated for each pair-wise comparisons between varietal treatments.

$$df = \frac{(var_1 + var_2)^2}{(var_1{}^2/N_1) + (var_2{}^2/N_2)}$$

Finally, a comparison between the degree of freedoms for the pair-wise comparison and the degree of freedom in the statistical tables was done, using t table= $t_{0.05\ (2)\ df\ calculated}$. If the t-statistic observed was higher than the one found in the statistic tables, then the null hypothesis was not accepted, and the conclusion was that the varietal treatments compared were not significantly different (Jayaraman 2000, Moreno 2001).

Results and Discussion

Presence of natural enemies in Colorado, Kansas, and Nebraska

Differences in the relative abundance of various natural enemy groups on barley plants at Fort Collins, Colorado; Tribune, Kansas and Sidney, Nebraska during 2007, 2008, and 2009 are presented in Fig. 4.1. In 2007, Hemiptera were significantly lower at Fort Collins, compared to Tribune or Sidney. The abundance of other major groups of aphid natural enemies was similar among states.

In 2008, the highest numbers of parasitic wasps, Coccinellidae and spiders and the lowest numbers of Hemiptera were observed at Tribune, but the lowest numbers of Hemiptera. These numbers were significantly higher than those at Fort Collins or Sidney. No significantl differences were observed in Neuropteran counts across locations.

In 2009, all groups of natural enemies were significantly more abundant at Tribune than at Fort Collins or Sidney. Climatic profiles for each location (Appendix C) appeared to be highly responsible for differences observed in RWA and natural enemy populations. The highest range of temperature was observed at Tribune (23°C), compared to Fort Collins (18°C-20°C) and Sidney (18°C-21°C). Precipitation was highest at Sidney, followed by Fort Collins, and Tribune. These particular sets of climatic conditions may affect natural enemies in a different way than aphids, depending upon the specific requirements for their survival and development. Climatic conditions such as drought that adversely affect the establishment of RWA and other cereal aphids ,(Tribune, 2007 and 2008) or high precipitation (Sidney), could also affect the colonization and establishment of natural enemies, due to low prey availability. If climatic conditions are good (Fort Collins across years), both aphid and natural enemy numbers should increase. However, as natural enemy numbers increases, the conflict between natural enemy major groups is expected to increase as well due to prey and/or space limitation. High natural enemy density normally increases with prey population increases, but this also could create conflict among predators competing for the same resource. Landscape effects may also explain variation in natural enemy density based on ecological relationships. If barley varieties are surrounded by other cereal crops, they would potentially offer suitable alternative niches to specialist natural enemies of cereal aphids. However, if the landscape corresponds to non cereal crops, then only generalist predators would be favored by this alternative niche.

Effect of planting date by location on natural enemy populations. In 2007, barley planted on the normal schedule had significantly higher populations of spiders and parasitoids than early-planted plots at Fort Collins (Fig. 4.2). At Tribune, coccinellids were significantly more abundant in plots planted normally. At Sidney, Nebraska, numbers of predaceous hemipterans and spiders were higher in the normal planting date plots. In 2008, planting date had little effect on enemy abundance. Only hemipteran populations at Sidney were more abundant in the normal-planted plots compared to those that were planted early (Fig. 4.3). In 2009, hemipterans were found in higher numbers in normal-planted barley in all three states, but no other group of natural enemies was affected by planting date (Fig. 4.4). A comparison of all states and all study years suggested that planting date has some effect on natural enemy abundance, and that hemipterans are affected to a greater degree than other aphid predators or parasitoids.

In general, natural enemy populations tended to be high in normal planted plots and a plausible explanation for this fact is the high density of aphid populations in this planting date regime, and in consequence high prey availability. As presented in Figs. 4.5-4.13, the comparison of mean aphids to mean numbers of parasitic wasps and predators by location in 2007-2009 showed that, parasitic wasp, predator, and total aphid populations were synchronous. With an increase in the aphid populations in normal planted plots (especially in Otis) populations of natural enemies increased as well. In that sense, a density dependent response between natural enemy and aphid populations is possible. However, in order to detect a pattern in the population dynamics of both aphid and natural enemy populations, a more detailed study is required, such as a field cage population dynamics study using different locations, planting dates and varieties.

Exclusion cages were used by Nechols and Harvey (1991), to study the impact of Russian wheat aphid natural enemies using total exclusion, partial exclusion, no natural enemies and uncaged plots. Natural enemies and poor host quality were shown to limit the density of Russian wheat aphid populations. Lee et al. (2005) evaluated the effectiveness of RWA predators and parasitoids using mechanical exclusion in winter wheat fields in southeastern Colorado, and showed *H. convergens,* and the generalist *Nabis* spp. to be the most abundant.

Effect of varietal treatment by location on the natural enemy major group populations. Tables 4.1-4.3 show the abundance and proportion of the major natural enemy groups at Fort Collins, Colorado; Tribune, Kansas; and Sidney, Nebraska in 2007, 2008 and 2009.

Spiders. There were not significant differences in the mean populations of the spider group at any of the three locations in any of the three years of data collected (Table 4.4).

Parasitic wasps. At Fort Collins, Colorado, mean populations of parasitoid wasps were significantly lower on Otis plants treated with thiametoxam than Otis untreated plants in 2007, 2008 and 2009 **(**Table 4.5). In addtion, there were no differences in the numbers of wasps on Otis, Sidney and Stneham in 29007 and 2008. The same difference in wasp populations between Otis treated - and Otis untreated plants observed at Fort Collins occurred at Tribune, Kansas in 2008 and 2009, but there were no other varietal differences at this location. There were no significant differences in mean parasitoid wasp populations between any varieties at Sidney, Nebraska.

An explanation to these observations is that the chemical protection offered by thiamethoxam is of short residual effect, which works only in the early stage of plant growth in order to act as a barrier against insect pest on the most critical stages of growth. Bosque-Perez and colleagues (2002) found that the most abundant primary hymenopteran parasitoids were *Diaeretillea rapae*, *Aphidius ervi* Haliday, *Aphidius avenaphis* (Fitch), and *Lysiphlebus testaceipes* (Cresson). In the present study, the families of parasitoid wasps identified were similar to these findings. However, a more detailed identification is required in order to determine parasitoid wasp species.

Coccinellidae. There was no consistent pattern among years, locations or varietal treatments with respect to abundance of lady beetles (Table 4.6). Population densities were low in most years and locations. Coccinellids were relatively more abundant, and significantly so, at Tribune in 2009 on the susceptible variety, Otis, where no insecticide was used compared to all other treatments. This same pattern was observed at Fort Collins in 2009 but at lower densities (Table 4.8). At Sidney, Nebraska, population levels of coccinellids were comparably low and similar to those at Fort Collins; however, there were no differences among varieties. Due to low population levels, the results in this study cannot be compared with previous studies. As previously reported in literature, several species of coccinellids had cosmopolitan distribution and both larvae and adults are very active feeders (Völkl et al. 2007). Findings presented by several studies (Elliott et al. 1998, Michels et al. 2001, Bosque-Perez et al. 2002), suggested that some of the most important species were *H. convergens*, and *C. septempunctata*. Bosque-Perez et al. (2002) did not find significant differences in adult or immature coccinellid densities between the resistant and susceptible genotypes.

Hemiptera. Mean population numbers of predaceous Hemiptera (Table 4.7) were uniformly low at all locations and in all three years. Variety appeared to have no effect on hemipteran numbers, with the exception of populations observed on Otis thiametoxam treated plants at Sidney, Nebraska in 2008, which were significantly lowere than Otis, Sidney or Stoneham plants.

Neuroptera. Mean populations of Neuroptera (Table 4.8) were very low across all locations and there were no significant differences in populations at Sidney, Neraska and Fort Collins, Colorado in 2007, 2008 or 2009. At Tribune, Kansas, significantly greater numbers of Neuroptera were observed on Sidney and Stoneham than on Otis or Otis thiametoxam treated plants in 2007, but in 2009, significantly greater numbers were observed on Otis, Sidney and Stoneham than on Otis treated plants.

RWA resistance in Sidney (Otis x STARS 9301B) and Stoneham (Otis x STARS 9577B) has previously been demonstrated by Mornhinweg et al. (1995, 999) and Bregitzer et al. (2003). Bregitzer et al. (2003) showed that RWA populations were similar on susceptible and resistant varieties under greenhouse conditions but different under field conditions, and suggested that differences in the field were because resistant plants do not roll their leaves after infestation, which subjects RWA to mortality from natural enemies and abiotic factors such as wind or rain. A more recent study, by Murugan et al. (2010) demonstrated that Sidney and Stoneham exhibit moderate levels of antibiosis (reduced intrinsic rate of increase) and tolerance (reduced root and shoot dry weight loss) to RWA biotypes 1 and 2. In general, Sidney and Stoneham exhibit intermediate to low levels of RWA infestation when compared with the susceptible variety Otis. This resistance effect in these RWA-resistant varieties may to some extent affect the presence of natural enemies that feed directly on the aphid. Similarities in the abundance of parasitic wasps among RWA-resistant and susceptible barley cultivars are in accordance with results found by

Brewer et al (1999), who observed that in field-grown barley lines with different levels of RWA resistance, parasitoid abundance was similar on resistant and susceptible barley lines. On the basis of these findings, they concluded that the use of resistant barley lines and RWA parasitoids were compatible.

Shannon-Wiener indices for barley varietal treatments

The second approach to understand how varieties may affect the abundance and diversity of natural enemies was to calculate the Shannon-Wiener index for each varietal treatment. Parasitic wasps consistently represented the largest group of aphid natural enemies (Fort Collins, CO mean range = 41-164; Tribune, KS mean range = 78-1,545; Sidney, NE mean range = 37-197 across varietal treatments). Within parasitoid wasps, specific aphid parasitoid families found across locations were Aphelinidae and Braconidae (including the genera *Ephedrus*, *Diaeretiella*, *Praon*, and *Lysiphlebus*). Within the Coccinellidae group, *Hippodamia convergens* Guèrin-Mèneville, *Coccinella septempunctata* L., *Hippodamia trifasciata* Mulsant were the main species. Unidentified coccinellid larvae were also present. The major Hemipteran predators were composed of the families Nabidae, Geocoridae and Anthocoridae. Finally, neuropteran specimens from the families Chrysopidae and Hemerobiidae were present but in less proportion compared to other major natural enemy groups.

Appendix D lists the arthropods found in each of the five major natural enemy groups, identified to the lowest possible taxonomic level. These data were used to compute a Shannon-Wiener diversity index (H’) and variance for each varietal treatment, which are show in Appendix E, along with pair-wise statistical comparisons of indices values of each varietal treatment combination. The natural enemy diversity index values on plants of Sidney and Stoneham at Fort Collins, differed significantly in 2007, but not in 2008 or 2009 (Table 4.9). There were no other significant differences in any pairwise comparisons at Fort Collins in any of the three years of study. The reason for these low index values could be due to the low occurrence of coccinellid and hemipteran predators in the sample.

At Tribune, the diversity index of Sidney plants was significantly lower than those on plants of Otis, Stoneham or Otis plants treated with thiamethoxam in 2007 and in 2008, the same trend was evident for plants of Stoneham, which had a diversity index significantly lower than plants of Otis, Sidney or Otis plants treated with thiamethoxam. In 2009 at Tribune, all pairwise of diversity indices were significant except the comparison between Sidney and Stoneham. The indices on plants of Otis with and without thiamethoxam were significantly different, and the index of each Otis treatment was significantly greater than the index on either Sidney or Stoneham plants. In general, these low index values implied reduced representation of spiders, coccinellid, and hemipteran predators. Drought stress was severe at Tribune in 2007 and 2008 (Chapter 2 and Appendix C) adversely affecting the crop, and reducing aphid and certain natural enemy populations. However, in 2009, when climatic conditions were extremely good for establishment of RWA and other cereal aphid populations, there was a high representation of parasitic wasps and a low representation of Coccinellidae. An extremely elevated density of aphid populations inhabiting the RWA-resistant varieties would be more easily detected by parasitic wasps, mainly because these varieties do not roll leaves as the susceptible variety, and parasitic wasps could more easily detect the presence of high aphid densities.

At Sidney, Nebraska, no differences in diversity indices were seen between any pair of varietal treatments in 2007. In 2008, Otis plants had a significantly higher index than either Otis treated with thiamethoxam of Sidney plants, but no index differences existed between plants of Otis and Stoneham. Parasitic wasps were highly represented and coccinellid, hemiptera and neuropteran predators were sparsely representated. In 2009, Sidney plants had a greater index than plants of either Otis or Stoneham and there was no difference in the diversity indices of Otis plants with- of without thiamethoxam. Index values for coccinellids and hemipteran predators were low on Sidney compared to Otis and Stoneham.

A trend observed in this research was a reduction in the Shannon-Wiener indices of the RWA-resistant barley varieties. As explained before, this reduction could be related to a high representation of fewer groups (e. g. parasitic wasps), or resistance factors in the host plant (e. g. antibiosis), adverse climatic conditions, ecological relationships between natural enemies or landscape effects.

A high abundance of fewer natural enemy groups such as parasitic wasps would be expected in RWA-resistant varieties, as explained above. Also, a host plant resistant trait such as antibiosis reduces aphid populations, and consequently the presence of natural enemies could also be affected. A reduction in natural enemies would be expected with the lack of suitable prey, reduction in prey density and reduced ability to search for scarce prey, but antibiosis can directly affect the biology of specialist natural enemies. Reed et al. (1992) studied the effect of the RWA-resistant wheat with antibiosis factor on the performance of the parasitic wasp *D. rapae*, and they found a prologantion of developmental time in this parasitic wasp when it developed on RWA reared on the resistant wheat. However, Farid et al. (1997) showed that total developmental time was not different from coccinellid predators that fed on aphids reared on susceptible ('Stephens') and resistant (PI 137739) wheat. Also, Bosque-Perez et al. (2002) did not find interaction between RWA-resistant genotypes and the population density of the predators or parasitoids that were monitored, including adults and immature coccinellids. Climatic conditions may also reduce diversity indices because specific requirements for temperature and abiotic factors change from one species to another and may limit the establishment and success of some natural enemies.

Conclusions

During the three years of study, cereal aphid infestation levels and natural enemies were highly variable within locations, because the climatic conditions in East Central Colorado, Western Kansas, and Western Nebraska are very different. The highest aphid and natural enemy populations occurred at Tribune, in Western Kansas, in 2009. Higher densities of cereal aphid populations in normal planted plots than early planted plots, and as a result, there was a higher abundance of natural enemies in normal planted plots. Information provided by the Shannon-Wiener diversity index showed that in general, the RWA-resistant barley varieties Sidney and Stoneham are not extremely different in the diversity of fauna they contain when compared to the susceptible variety, Otis. In some of the cases, index values on the resistant varieties were higher than on Otis. RWA-resistant barley varieties may have different effects on the performance of major groups of natural enemies and further research would be require to assess the impact of the antibiosis factor on the biology of natural enemies. In certain years and locations, Otis plants treated with thiametoxam had lower diversity index values that could be due to a reduction in aphid populations. The short residual effect of thiametoxam, which works in the early stage of plant growth against insect pests, may reduce aphid availability and therefore reducing the diversity values. Also, the analysis of variance indicated location to be the main factor causing such differences in diversity values. Further studies are necessary in order to assess the potential effects of antibiosis and the effect of the systemic insecticide on the biology of natural enemies.

Results for the effect of planting dates were not definitive, but the general trend observed was the presence of higher mean populations of natural enemies on plants in normal planting date plots. However, most differences were not statistically significant.

There were no differences in spider, Hemiptera, and Neuroptera populations were found between varieties within locations. There were no clear differences in mean parasitoid wasp populations on plants of RWA-resistant varieties compared to the susceptible variety Otis. In some cases, populations were lower on Otis plants treated with thiametoxam. Finally, Coccinellidae mean populations were generally higher on Otis plants, but again, no clear differences were observed between populations on other varieties across locations and years.

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Tables and Figures

Table 4.1 Abundance of major natural enemy groups on Otis, Sidney and Stoneham barley varieties at Fort Collins, Colorado; Tribune, Kansas and Sidney, Nebraska in 2007

Location	Major groups	Otis		Otis_p		Sidney		Stoneham	
		n_i	p_i	n_i	p_i	n_i	p_i	n_i	p_i
Colorado	Spiders	9	0.11	17	0.32	16	0.22	17	0.26
	Parasitoid wasps (PW)	58	0.73	26	0.49	38	0.51	41	0.63
	Coccinellidae	3	0.04	4	0.08	13	0.18	3	0.05
	Hemiptera predators	9	0.11	6	0.11	7	0.09	3	0.05
	Neuroptera	0	0.00	0	0.00	0	0.00	1	0.02
	Total	**79**		**53**		**74**		**65**	
Kansas	Spiders	13	0.10	14	0.11	18	0.13	16	0.16
	Parasitoid wasps	83	0.67	83	0.67	94	0.65	54	0.56
	Coccinellidae	7	0.06	12	0.10	9	0.06	10	0.10
	Hemiptera predators	19	0.15	14	0.11	16	0.11	13	0.13
	Neuroptera	2	0.02	1	0.01	7	0.05	4	0.04
	Total	**124**		**124**		**144**		**97**	
Nebraska	Spiders	13	0.18	12	0.18	24	0.23	11	0.16
	Parasitoid wasps	38	0.51	34	0.50	47	0.46	32	0.46
	Coccinellidae	5	0.07	4	0.06	4	0.04	8	0.11
	Hemiptera predators	17	0.23	17	0.25	28	0.27	19	0.27
	Neuroptera	1	0.01	1	0.01	0	0.00	0	0.00
	Total	**74**		**68**		**103**		**70**	

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

n_i= Number of individuals collected in each major group by variety

p_i = Relative proportion of individuals in each major group with respect with total individuals collected on each variety by location

Table 4.2 Abundance of major natural enemy groups on Otis, Sidney and Stoneham barley varieties at Fort Collins, Colorado; Tribune, Kansas and Sidney, Nebraska in 2008

Location	Major groups	Otis		Otis_p		Sidney		Stoneham	
		n_i	p_i	n_i	p_i	n_i	p_i	n_i	p_i
Colorado	Spiders	8	0.07	3	0.05	3	0.03	3	0.03
	Parasitoid wasps (PW)	71	0.62	28	0.50	49	0.50	61	0.56
	Coccinellidae	9	0.08	6	0.11	14	0.14	15	0.14
	Hemiptera predators	24	0.21	19	0.34	31	0.32	30	0.28
	Neuroptera	2	0.02	0	0.00	1	0.01	0	0.00
	Total	**114**		**56**		**98**		**109**	
Kansas	Spiders	27	0.10	21	0.14	23	0.12	31	0.09
	Parasitoid wasps	195	0.74	96	0.62	134	0.71	267	0.78
	Coccinellidae	35	0.13	28	0.18	25	0.13	32	0.09
	Hemiptera predators	6	0.02	7	0.05	8	0.04	6	0.02
	Neuroptera	2	0.01	2	0.01	0	0.00	5	0.01
	Total	**265**		**154**		**190**		**341**	
Nebraska	Spiders	10	0.06	6	0.06	5	0.04	5	0.04
	Parasitoid wasps	105	0.66	79	0.84	88	0.68	93	0.65
	Coccinellidae	17	0.11	2	0.02	4	0.03	16	0.11
	Hemiptera predators	22	0.14	7	0.07	30	0.23	27	0.19
	Neuroptera	5	0.03	0	0.00	2	0.02	1	0.01
	Total	**159**		**94**		**129**		**142**	

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

n_i= Number of individuals collected in each major group by variety

p_i = Relative proportion of individuals in each major group with respect with total individuals collected on each variety by location

Table 4.3 Abundance of major natural enemy groups on Otis, Sidney and Stoneham barley varieties at Fort Collins, Colorado; Tribune, Kansas and Sidney, Nebraska in 2009

Location	Major groups	Otis		Otis_p		Sidney		Stoneham	
		n_i	p_i	n_i	p_i	n_i	p_i	n_i	p_i
Colorado	Spiders	3	0.01	5	0.04	3	0.02	4	0.02
	Parasitoid wasps (PW)	246	0.87	104	0.85	142	0.85	166	0.89
	Coccinellidae	26	0.09	3	0.02	15	0.09	4	0.02
	Hemiptera predators	6	0.02	9	0.07	3	0.02	10	0.05
	Neuroptera	2	0.01	1	0.01	4	0.02	2	0.01
	Total	**283**		**122**		**167**		**186**	
Kansas	Spiders	12	0.01	12	0.01	11	0.01	19	0.01
	Parasitoid wasps	1,677	0.78	1,138	0.82	1,790	0.87	1,577	0.85
	Coccinellidae	295	0.14	148	0.11	141	0.07	125	0.07
	Hemiptera predators	114	0.05	79	0.06	100	0.05	98	0.05
	Neuroptera	42	0.02	15	0.01	24	0.01	28	0.02
	Total	**2,140**		**1,392**		**2,066**		**1,847**	
Nebraska	Spiders	7	0.03	6	0.03	6	0.03	6	0.03
	Parasitoid wasps	239	0.90	170	0.91	176	0.90	202	0.85
	Coccinellidae	6	0.02	3	0.02	4	0.02	11	0.05
	Hemiptera predators	13	0.05	7	0.04	5	0.03	13	0.05
	Neuroptera	2	0.01	1	0.01	4	0.02	5	0.02
	Total	**267**		**187**		**195**		**237**	

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

n_i= Number of individuals collected in each major group by variety

p_i = Relative proportion of individuals in each major group with respect with total individuals collected on each variety by location

Table 4.4 Mean numbers of spiders on Otis, Sidney and Stoneham barley varieties grown at Fort Collins Colorado; Tribune, Kansas and Sidney, Nebraska in 2007, 2008 and 2009

Location	Variety [a]	2007 Mean ± S. E. [b]	2008 Mean ± S. E.	2009 Mean ± S. E.
CO	Otis	0.6 ± 0.20 a	0.5 ± 0.11 a	0.2 ± 0.10 a
	Otis_p	1.1 ± 0.38 a	0.2 ± 0.04 a	0.3 ± 0.15 a
	Sidney	1.0 ± 0.33 a	0.2 ± 0.06 a	0.2 ± 0.10 a
	Stoneham	1.1 ± 0.38 a	0.2 ± 0.04 a	0.3 ± 0.11 a
KS	Otis	0.8 ± 0.34 a	1.7 ± 0.14 a	0.8 ± 0.19 a
	Otis_p	0.8 ± 0.26 a	1.3 ± 0.10 a	0.8 ± 0.25 a
	Sidney	1.1 ± 0.33 a	1.4 ± 0.05 a	0.7 ± 0.18 a
	Stoneham	1.0 ± 0.32 a	1.9 ± 0.18 a	1.2 ± 0.23 a
NE	Otis	0.8 ± 0.25 a	0.6 ± 0.05 a	0.4 ± 0.22 a
	Otis_p	0.8 ± 0.21 a	0.4 ± 0.06 a	0.4 ± 0.13 a
	Sidney	1.5 ± 0.38 a	0.3 ± 0.06 a	0.4 ± 0.18 a
	Stoneham	0.7 ± 0.18 a	0.3 ± 0.06 a	0.4 ± 0.15 a

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Means followed by the same letter within each column are not significantly different (pr<|t| = 0.05)

Table 4.5 Mean numbers of parasitic wasps (PW) on Otis, Sidney and Stoneham barley varieties grown at Fort Collins Colorado; Tribune, Kansas and Sidney, Nebraska in 2007, 2008 and 2009

Location	Variety [a]	2007 Mean ± S. E. [b]				2008 Mean ± S. E.				2009 Mean ± S. E.			
CO	Otis	3.6	±	0.72	a	4.5	±	0.24	a	15.4	±	1.67	a
	Otis_p	1.6	±	0.46	b	1.8	±	0.17	b	6.5	±	1.17	b
	Sidney	2.4	±	0.46	a	3.1	±	0.29	ab	8.9	±	1.46	b
	Stoneham	2.6	±	0.72	a	3.8	±	0.26	a	10.4	±	1.14	ab
KS	Otis	5.2	±	1.00	a	12.2	±	0.67	a	104.8	±	7.25	a
	Otis_p	5.2	±	1.44	a	6.0	±	0.45	b	71.1	±	9.03	b
	Sidney	5.9	±	1.60	a	8.4	±	0.45	ab	111.9	±	10.10	a
	Stoneham	3.4	±	0.68	a	16.7	±	2.42	a	98.6	±	7.90	a
NE	Otis	2.4	±	0.45	a	6.9	±	0.50	a	14.9	±	2.64	a
	Otis_p	2.1	±	0.60	a	4.9	±	0.24	a	10.6	±	1.27	a
	Sidney	2.9	±	0.40	a	5.6	±	0.26	a	11.0	±	1.45	a
	Stoneham	2.0	±	0.41	a	6.1	±	0.38	a	12.6	±	2.44	a

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Means followed by the same letter within each column are not significantly different (pr<|t| = 0.05)

Table 4.6 Mean numbers of Coccinellidae on Otis, Sidney and Stoneham barley varieties grown at Fort Collins Colorado; Tribune, Kansas and Sidney, Nebraska in 2007, 2008 and 2009

Location	Variety [a]	2007 Mean ± S. E. [b]	2008 Mean ± S. E.	2009 Mean ± S. E.
CO	Otis	0.3 ± 0.15 b	0.7 ± 0.10 a	1.7 ± 0.42 a
	Otis_p	0.3 ± 0.11 b	0.4 ± 0.09 a	0.3 ± 0.14 b
	Sidney	1.0 ± 0.24 a	1.0 ± 0.16 a	0.9 ± 0.32 b
	Stoneham	0.3 ± 0.14 b	1.0 ± 0.14 a	0.3 ± 0.12 b
KS	Otis	0.5 ± 0.18 a	2.3 ± 0.19 a	18.4 ± 3.44 a
	Otis_p	0.9 ± 0.26 a	1.8 ± 0.16 a	9.4 ± 1.63 b
	Sidney	0.8 ± 0.21 a	1.8 ± 0.14 a	8.9 ± 1.07 b
	Stoneham	0.7 ± 0.25 a	2.0 ± 0.22 a	7.9 ± 1.15 b
NE	Otis	0.3 ± 0.12 a	1.1 ± 0.12 a	0.9 ± 0.23 a
	Otis_p	0.4 ± 0.13 a	0.2 ± 0.04 b	1.0 ± 0.56 a
	Sidney	0.3 ± 0.18 a	0.3 ± 0.06 b	0.6 ± 0.24 a
	Stoneham	0.6 ± 0.32 a	1.0 ± 0.11 a	1.4 ± 0.61 a

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Means followed by the same letter within each column are not significantly different (pr<|t| = 0.05)

Table 4.7 Mean numbers of hemipteran predators on Otis, Sidney and Stoneham barley varieties grown at Fort Collins Colorado; Tribune, Kansas and Sidney, Nebraska in 2007, 2008 and 2009

Location	Variety [a]	2007 Mean ± S. E. [b]	2008 Mean ± S. E.	2009 Mean ± S. E.
CO	Otis	0.6 ± 0.27 a	1.5 ± 0.11 a	0.4 ± 0.15 a
	Otis_p	0.4 ± 0.13 a	1.2 ± 0.14 a	0.6 ± 0.24 a
	Sidney	0.4 ± 0.18 a	1.9 ± 0.16 a	0.2 ± 0.14 a
	Stoneham	0.2 ± 0.10 a	1.9 ± 0.15 a	0.6 ± 0.20 a
KS	Otis	1.2 ± 0.42 a	0.4 ± 0.05 a	7.1 ± 0.86 a
	Otis_p	0.9 ± 0.22 a	0.4 ± 0.06 a	4.9 ± 0.83 a
	Sidney	1.0 ± 0.35 a	0.5 ± 0.08 a	6.3 ± 1.12 a
	Stoneham	0.8 ± 0.26 a	0.4 ± 0.06 a	6.1 ± 0.58 a
NE	Otis	1.1 ± 0.31 a	1.4 ± 0.13 a	0.8 ± 0.21 a
	Otis_p	1.1 ± 0.40 a	0.4 ± 0.06 b	0.4 ± 0.20 a
	Sidney	1.8 ± 0.49 a	1.9 ± 0.19 a	0.3 ± 0.12 a
	Stoneham	1.2 ± 0.42 a	1.7 ± 0.17 a	0.8 ± 0.33 a

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Means followed by the same letter within each column are not significantly different (pr<|t| = 0.05)

Table 4.8 Mean numbers of neuropteran predators on Otis, Sidney and Stoneham barley varieties grown at Fort Collins Colorado; Tribune, Kansas and Sidney, Nebraska in 2007, 2008 and 2009

Location	Variety [a]	2007 Mean ± S. E. [b]		2008 Mean ± S. E.		2009 Mean ± S. E.	
CO	Otis	0.0 ± 0.00	a	0.1 ± 0.03	a	0.1 ± 0.09	a
	Otis_p	0.0 ± 0.00	a	0.0 ± 0.00	a	0.1 ± 0.06	a
	Sidney	0.0 ± 0.00	a	0.1 ± 0.03	a	0.3 ± 0.14	a
	Stoneham	0.1 ± 0.06	a	0.0 ± 0.00	a	0.1 ± 0.09	a
KS	Otis	0.1 ± 0.09	b	0.1 ± 0.03	a	2.6 ± 0.48	a
	Otis_p	0.1 ± 0.06	b	0.1 ± 0.05	a	0.9 ± 0.25	c
	Sidney	0.4 ± 0.18	a	0.0 ± 0.00	a	1.5 ± 0.37	b
	Stoneham	0.3 ± 0.11	ab	0.3 ± 0.08	a	1.8 ± 0.32	ab
NE	Otis	0.1 ± 0.06	a	0.3 ± 0.05	a	0.1 ± 0.09	a
	Otis_p	0.1 ± 0.06	a	0.0 ± 0.00	a	0.1 ± 0.06	a
	Sidney	0.0 ± 0.00	a	0.1 ± 0.03	a	0.3 ± 0.14	a
	Stoneham	0.0 ± 0.00	a	0.1 ± 0.03	a	0.3 ± 0.15	a

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Means followed by the same letter within each column are not significantly different (pr<|t| = 0.05)

Table 4.9 Shannon-Wiener (H) index values for Otis, Sidney and Stoneham barley varieties grown at Fort Collins Colorado; Tribune, Kansas and Sidney, Nebraska in 2007, 2008 and 2009

Year	Variety [a]	H indices[b]					
		Colorado		Kansas		Nebraska	
2007	Otis	0.80	ab	0.89	a	0.96	a
	Otis_p	0.80	ab	0.87	a	0.87	a
	Sidney	0.93	a	0.70	b	0.87	a
	Stoneham	0.75	b	0.91	a	0.88	a
2008	Otis	0.88	a	0.77	a	0.87	a
	Otis_p	0.89	a	0.81	a	0.70	b
	Sidney	0.95	a	0.78	a	0.71	b
	Stoneham	0.87	a	0.60	b	0.80	a
2009	Otis	0.75	a	0.65	a	0.69	a
	Otis_p	0.78	a	0.60	b	0.63	ab
	Sidney	0.76	a	0.50	c	0.61	b
	Stoneham	0.75	a	0.51	c	0.77	a

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Means followed by the same letter within each column are not significantly different (pr<|t| = 0.05). Mean separation based on t-statistic (t) calculated for each pair-wise comparison between varietal treatments by location and year.

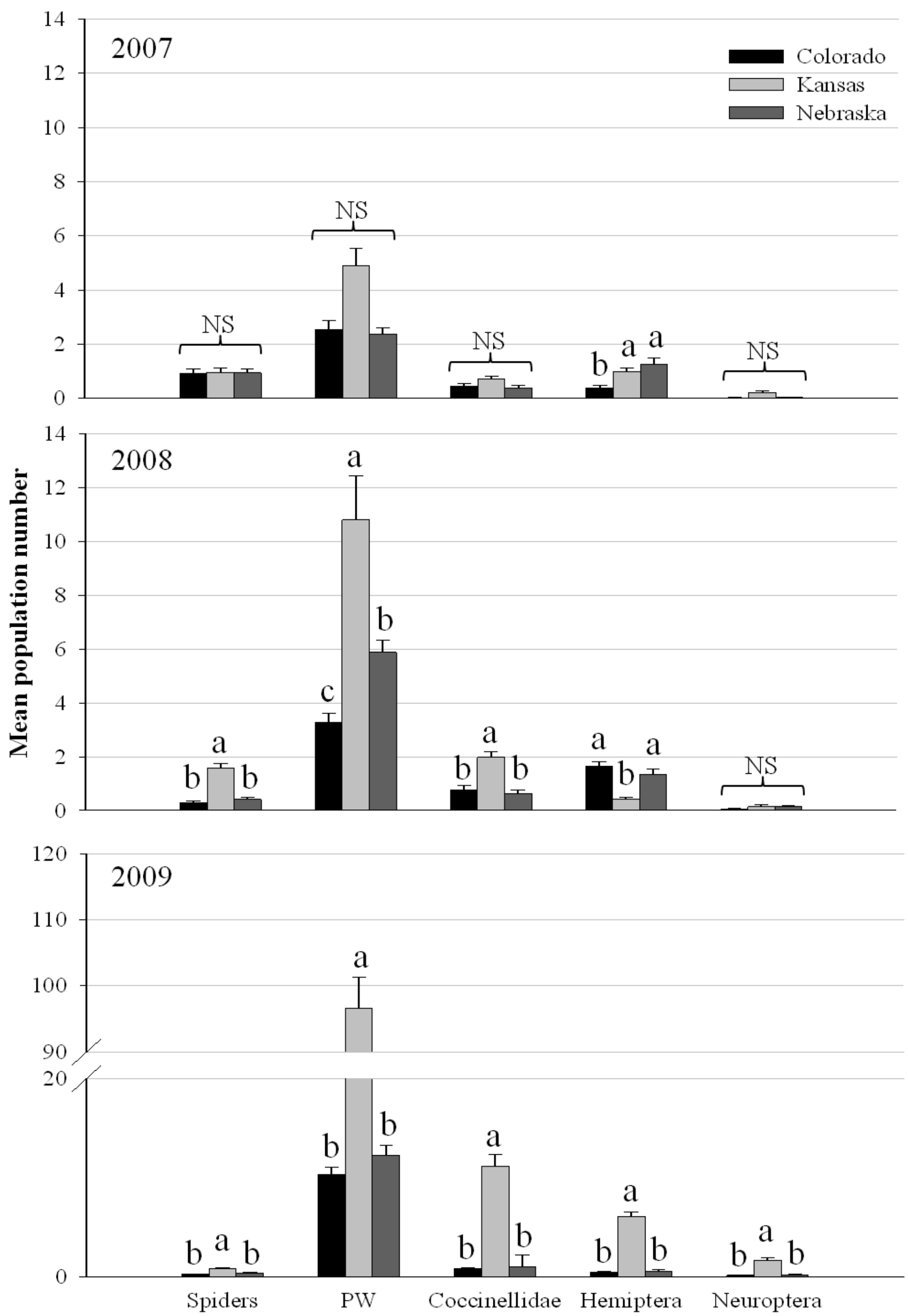


Fig. 4.1 Mean natural enemy populations on barley varieties grown at Fort Collins Colorado; Tribune, Kansas and Sidney, Nebraska in 2007, 2008 and 2009.

PW = parastioid wasps.

Means followed by the same letter within each column are not significantly different (pr<|t| = 0.05).NS = non significant differences

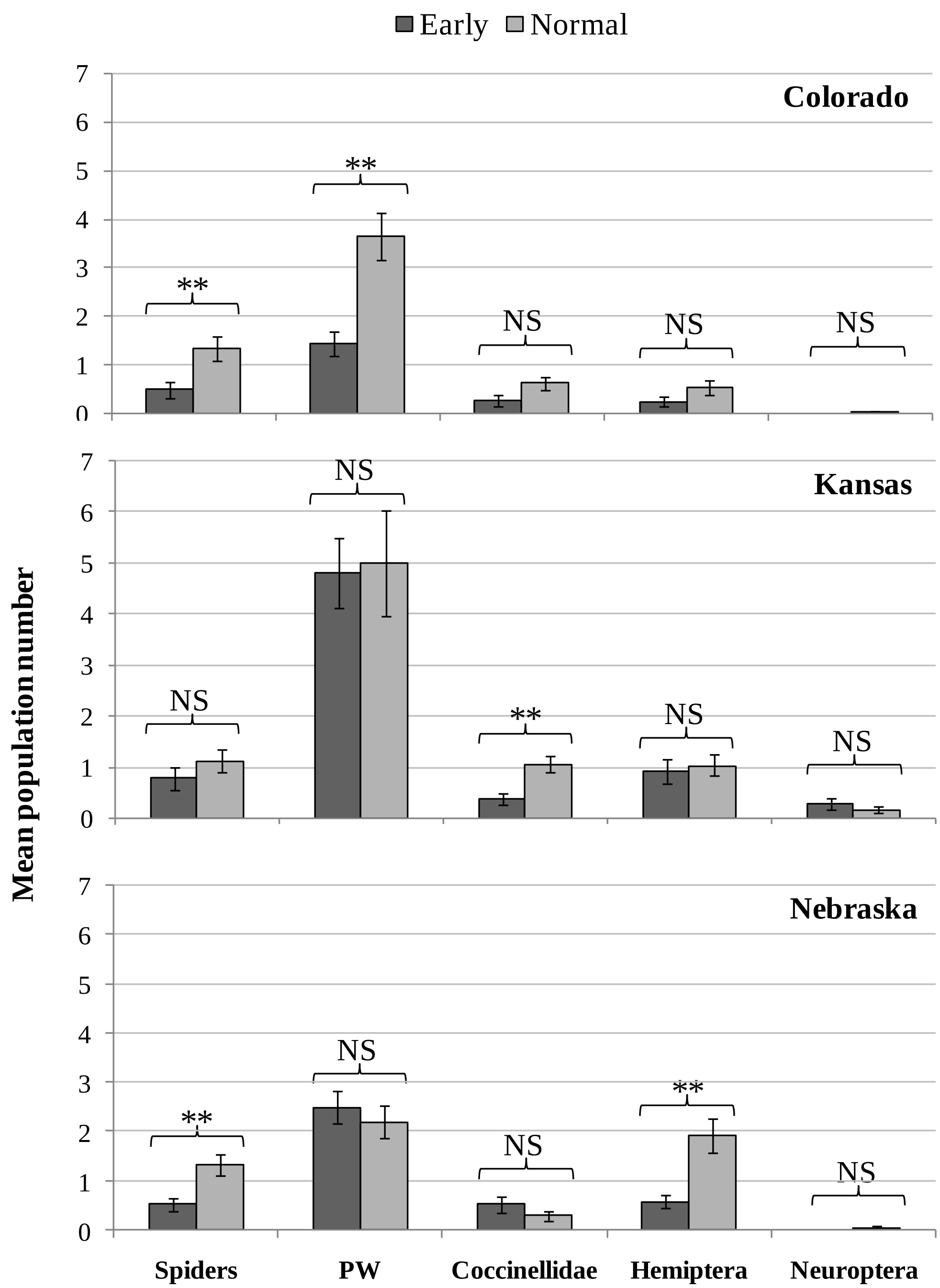


Fig. 4.2 Differences in natural enemy populations on early- and normal planted barley varieties grown at Fort Collins Colorado; Tribune, Kansas and Sidney, Nebraska in 2007.

PW= Parasitoid wasps

** Significant at 1%, * significant at 5%, NS = non significant difference

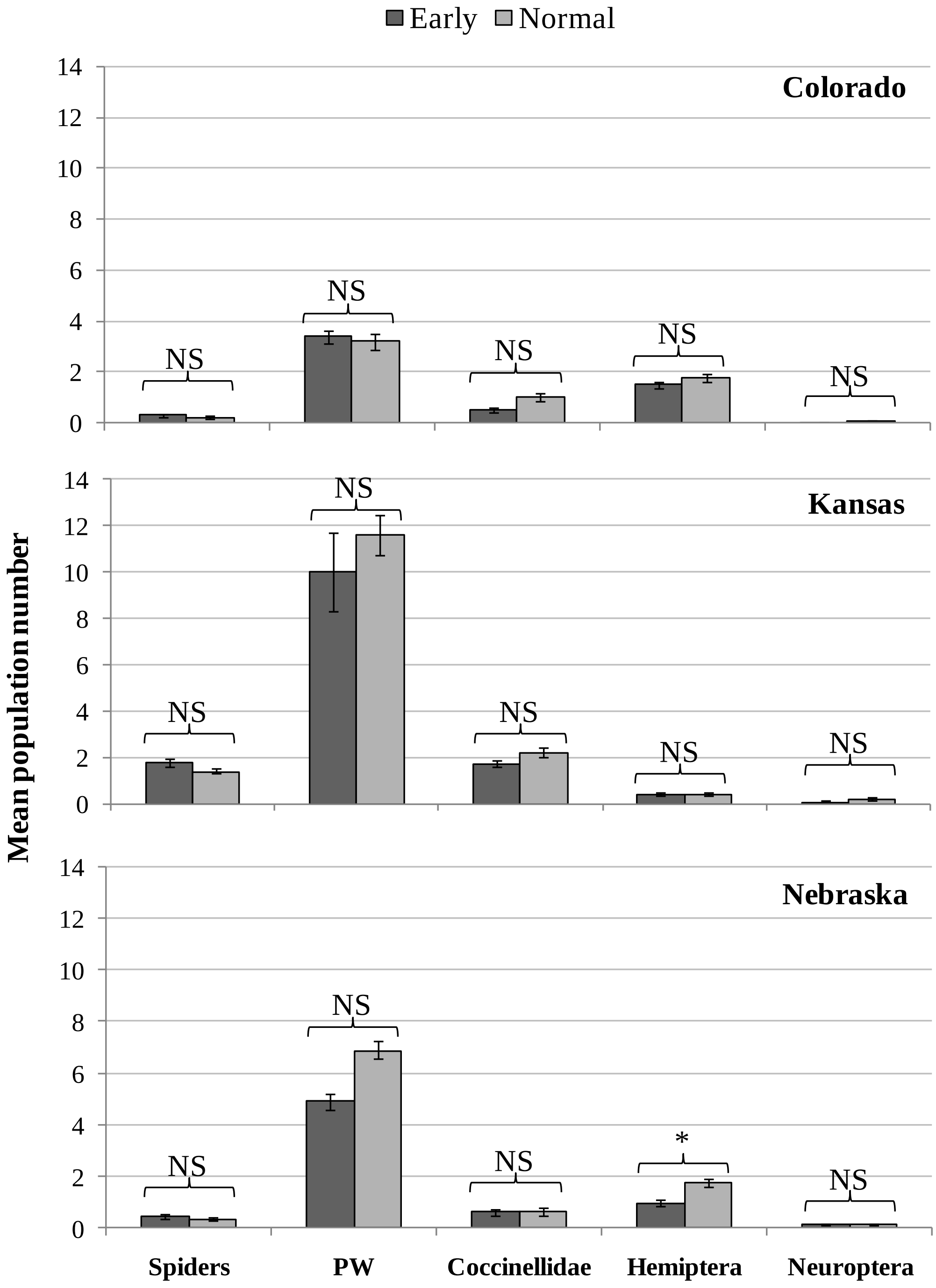


Fig. 4.3 Differences in natural enemy populations on early- and normal planted barley varieties grown at Fort Collins Colorado; Tribune, Kansas and Sidney, Nebraska in 2008.

PW= Parasitoid wasps

** Significant at 1%, * significant at 5%, NS= non significant difference

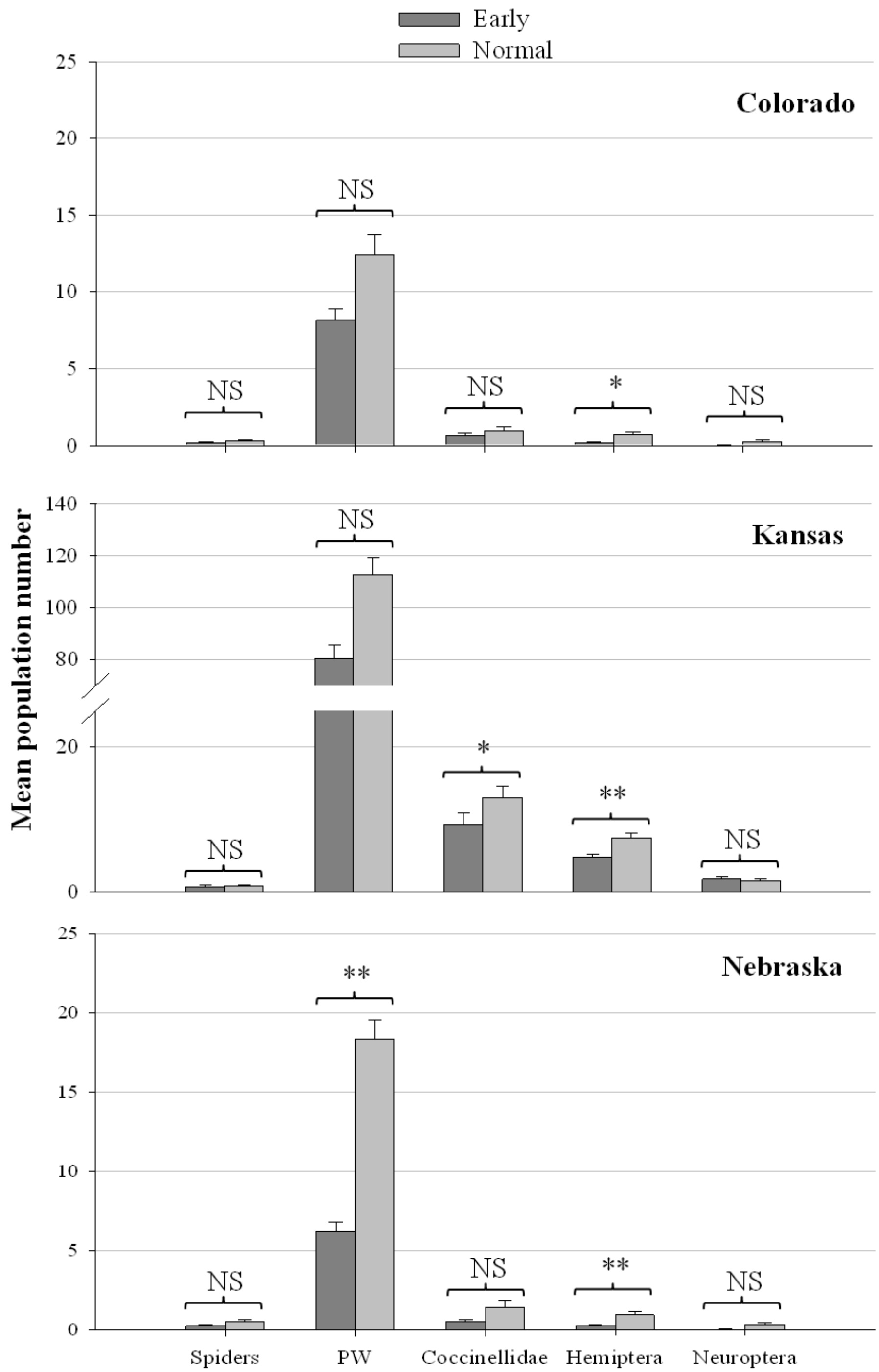
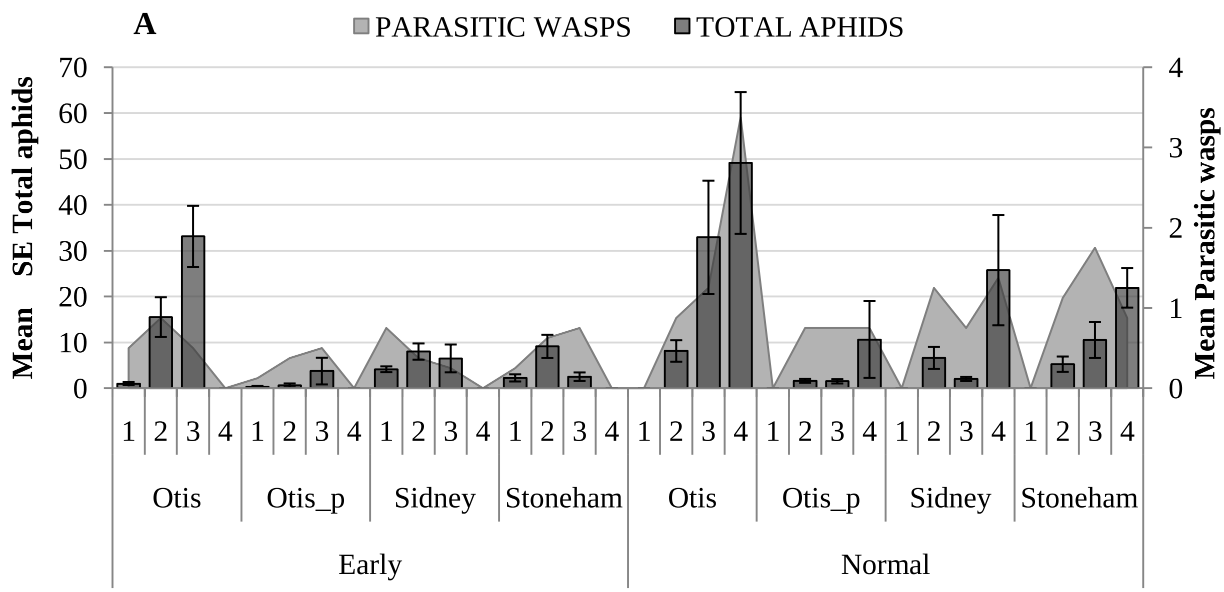


Fig. 4.4 Differences in natural enemy populations on early- and normal planted barley varieties grown at Fort Collins Colorado; Tribune, Kansas and Sidney, Nebraska in 2008.

PW= Parasitoid wasps

** Significant at 1%, * significant at 5%, n. s. = non significant difference



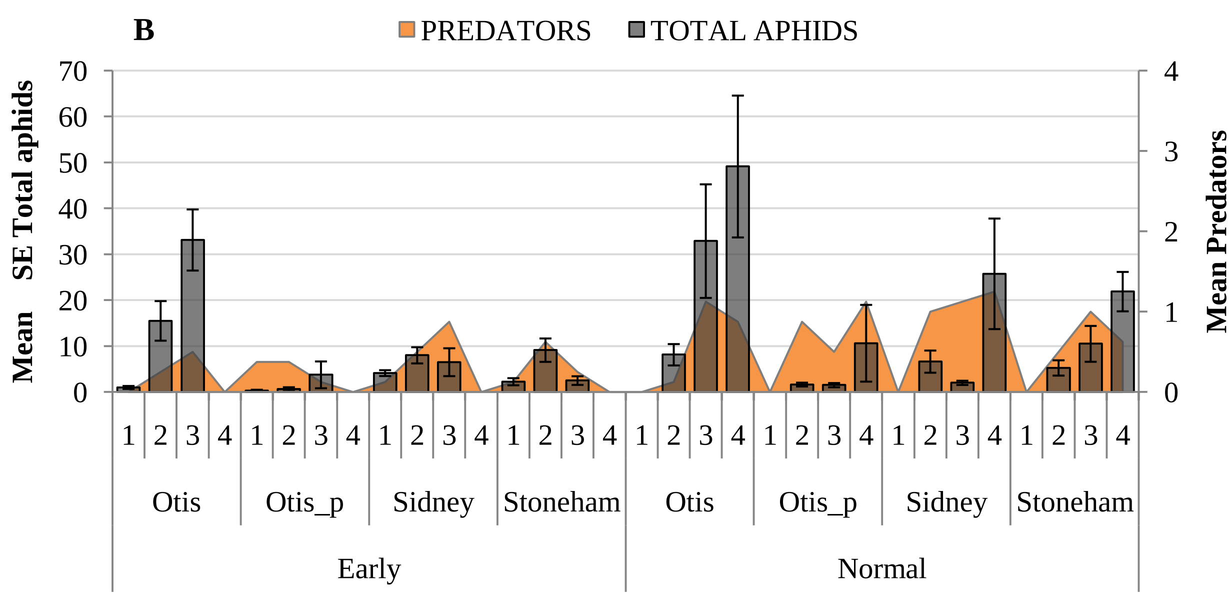
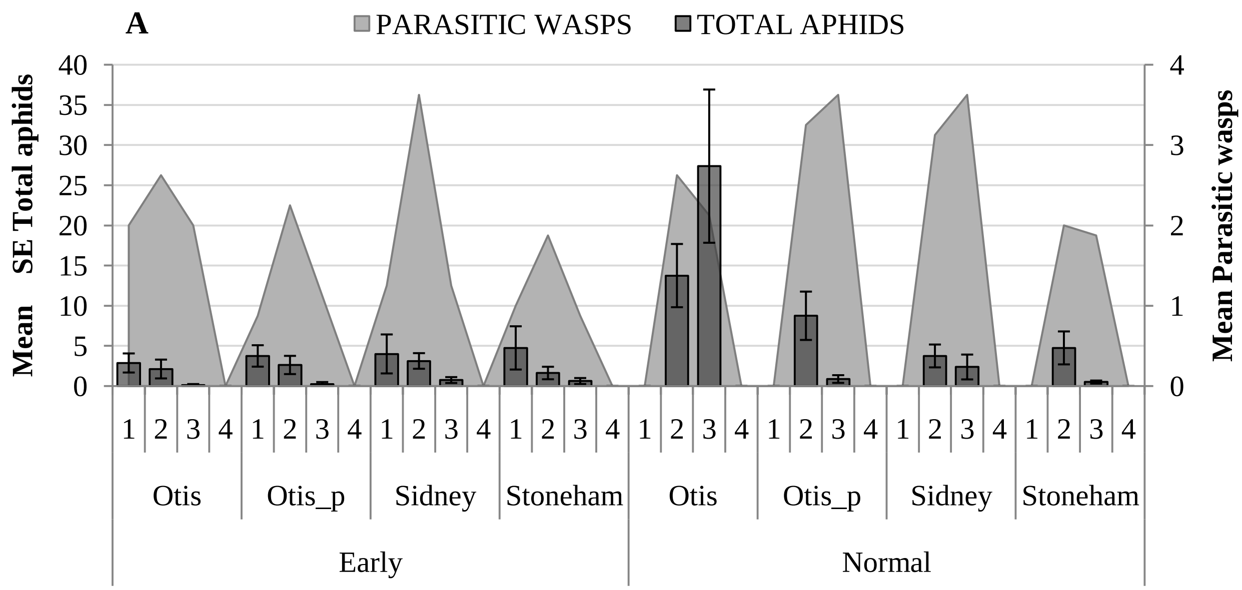


Fig. 4.5 Comparison of mean ± SE Total aphids to mean numbers of A) Parasitic wasps and B) Predators, by planting date, sampling date, and variety, in Colorado in 2007.

Otis = Susceptible variety, Otis_p = Otis under thiametoxam regime, Resistant varieties = Sidney and Stoneham.



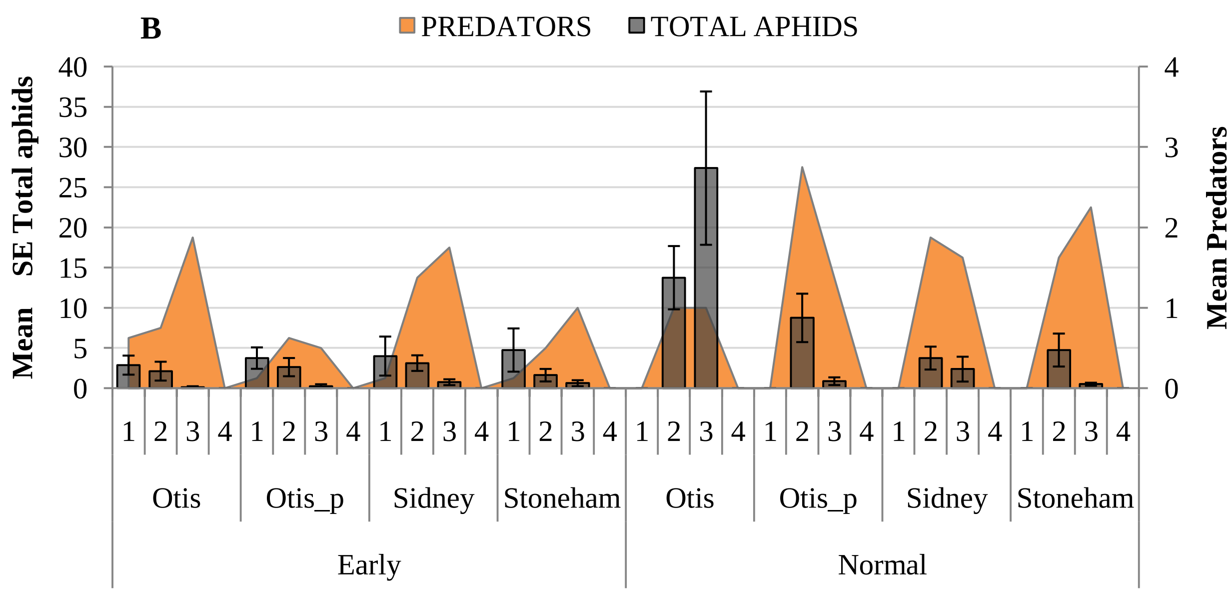


Fig. 4.6 Comparison of mean ± SE Total aphids to mean numbers of A) Parasitic wasps and B) Predators, by planting date, sampling date, and variety, in Kansas in 2007.

Otis = Susceptible variety, Otis_p = Otis under thiametoxam regime, Resistant varieties = Sidney and Stoneham.

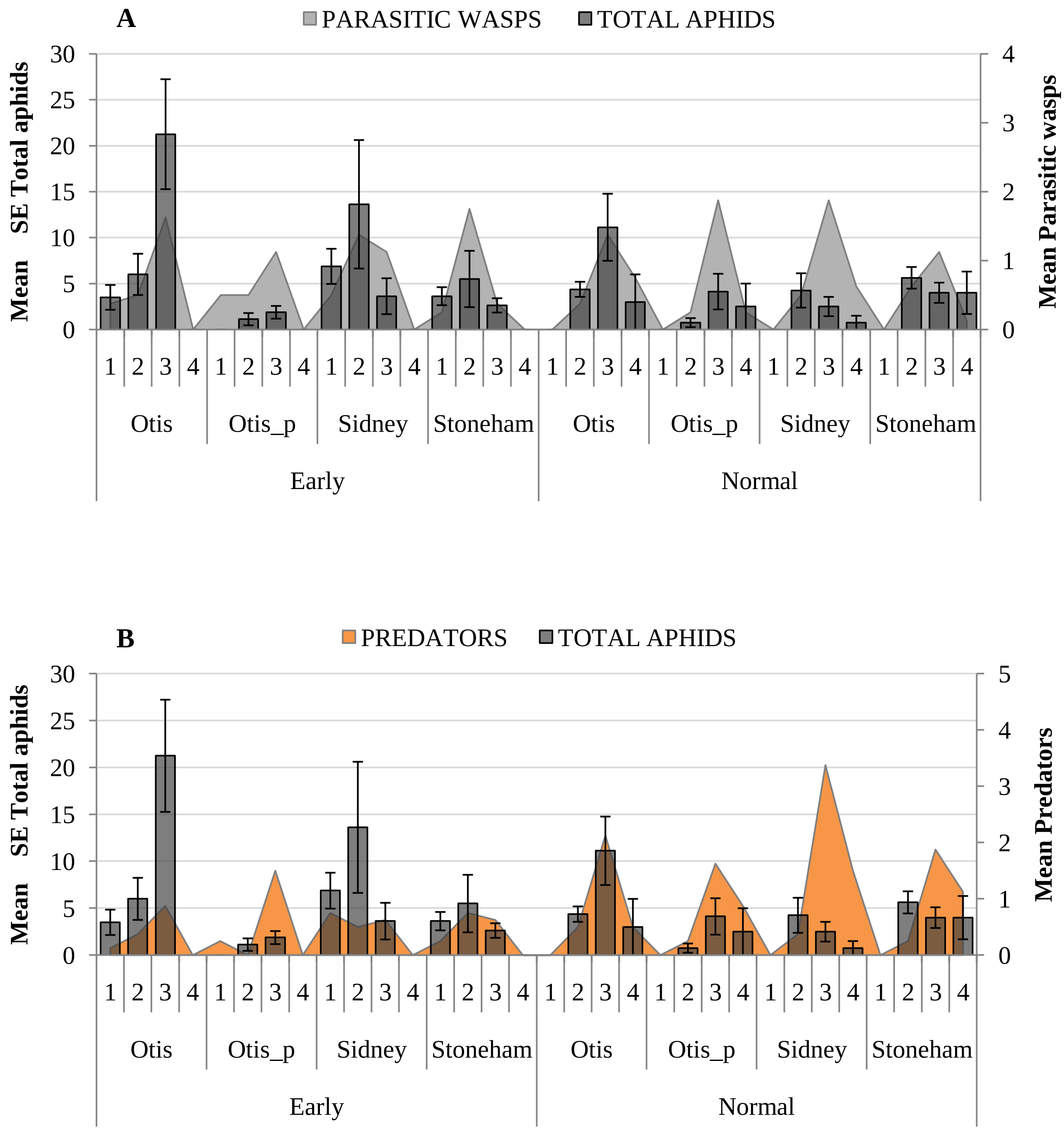
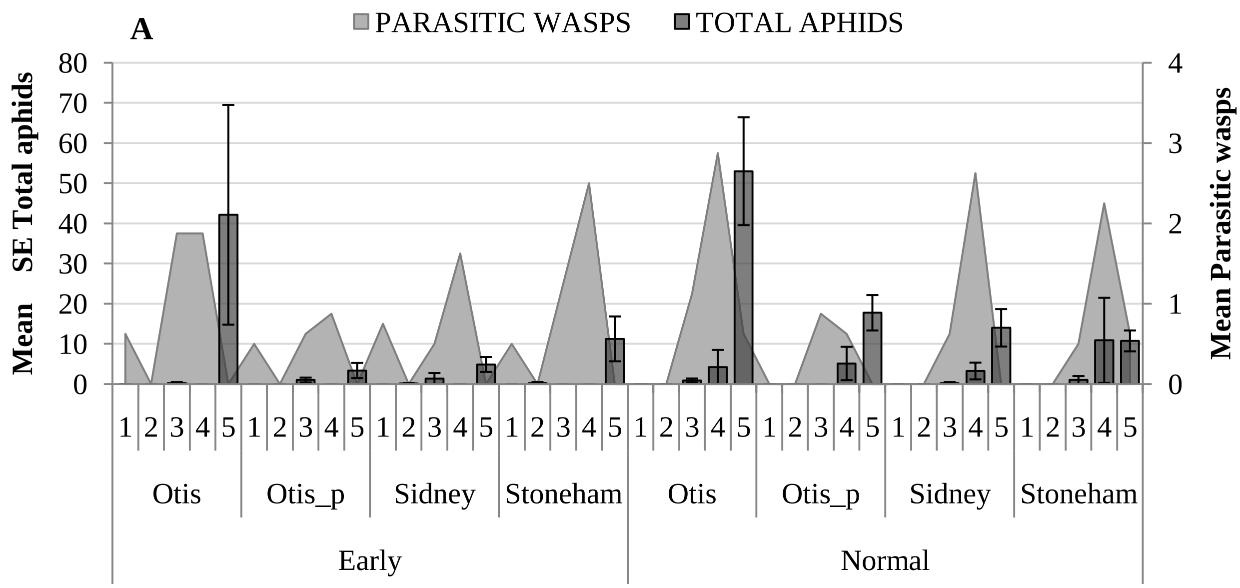


Fig. 4.7 Comparison of mean ± SE Total aphids to mean numbers of A) Parasitic wasps and B) Predators, by planting date, sampling date, and variety, in Nebraska in 2007.

Otis = Susceptible variety, Otis_p = Otis under thiametoxam regime, Resistant varieties = Sidney and Stoneham.



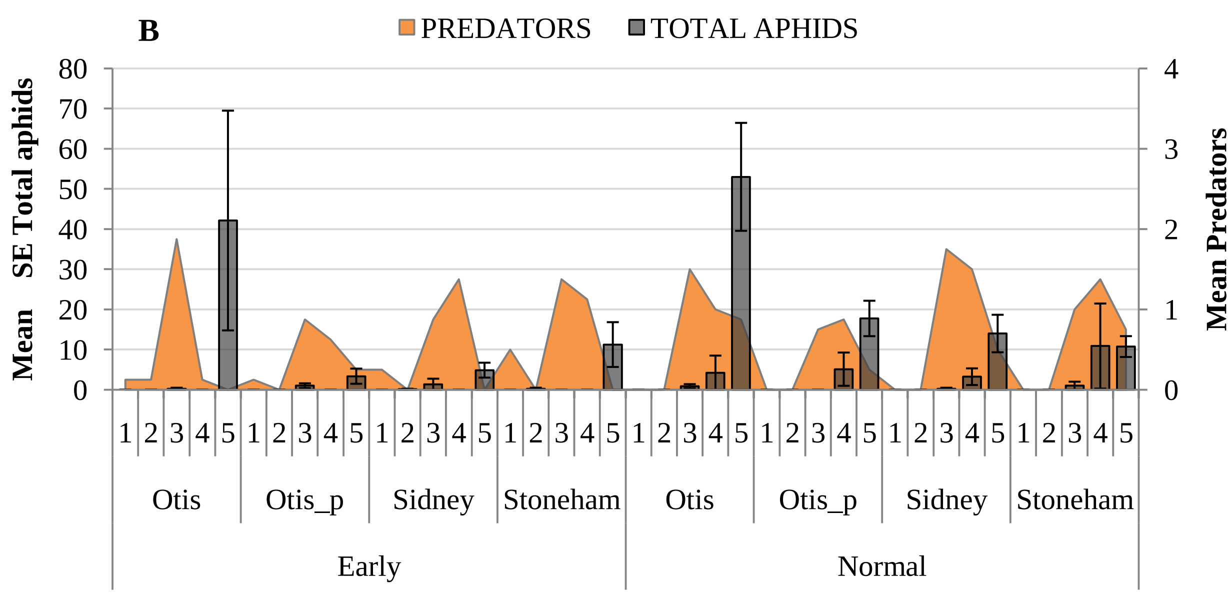


Fig. 4.8 Comparison of mean ± SE Total aphids to mean numbers of A) Parasitic wasps and B) Predators, by planting date, sampling date, and variety, in Colorado in 2008.

Otis = Susceptible variety, Otis_p = Otis under thiametoxam regime, Resistant varieties = Sidney and Stoneham.

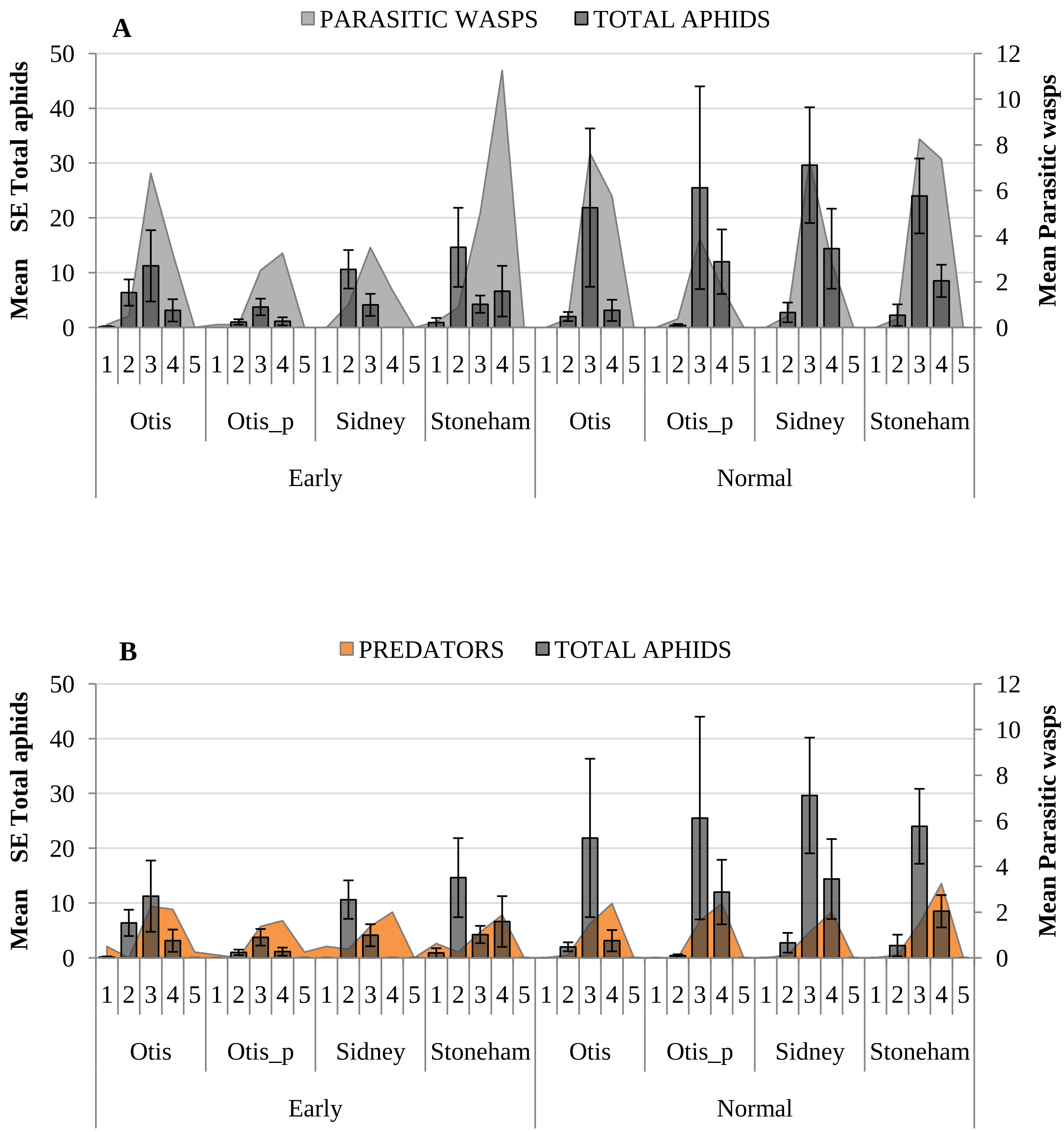
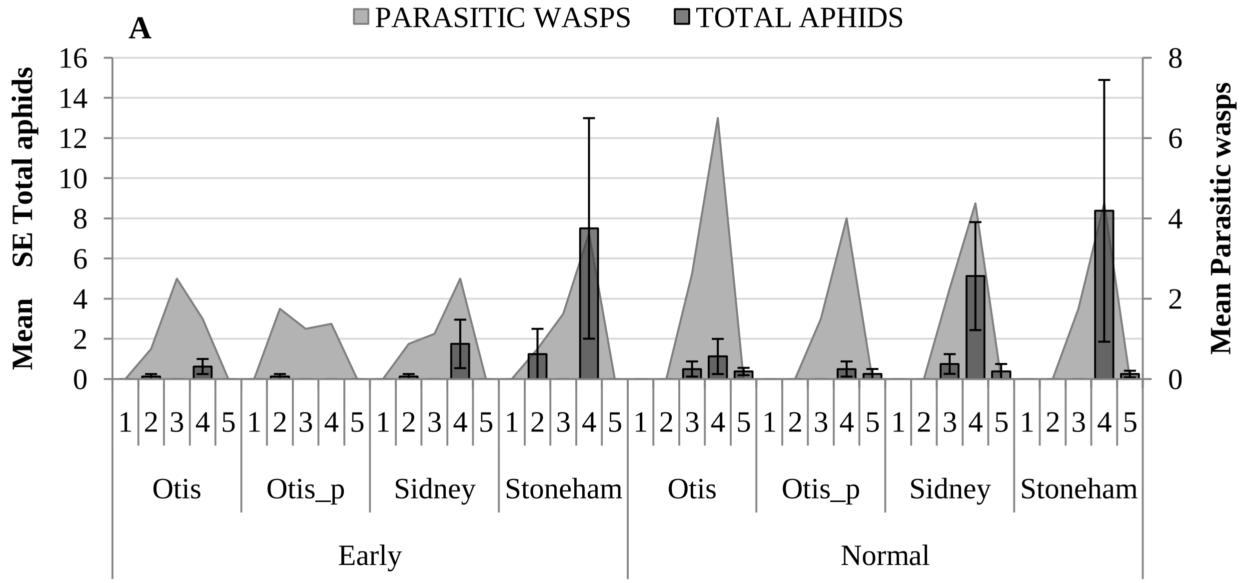


Fig. 4.9 Comparison of mean ± SE Total aphids to mean numbers of A) Parasitic wasps and B) Predators, by planting date, sampling date, and variety, in Kansas in 2008.

Otis = Susceptible variety, Otis_p = Otis under thiametoxam regime, Resistant varieties = Sidney and Stoneham.



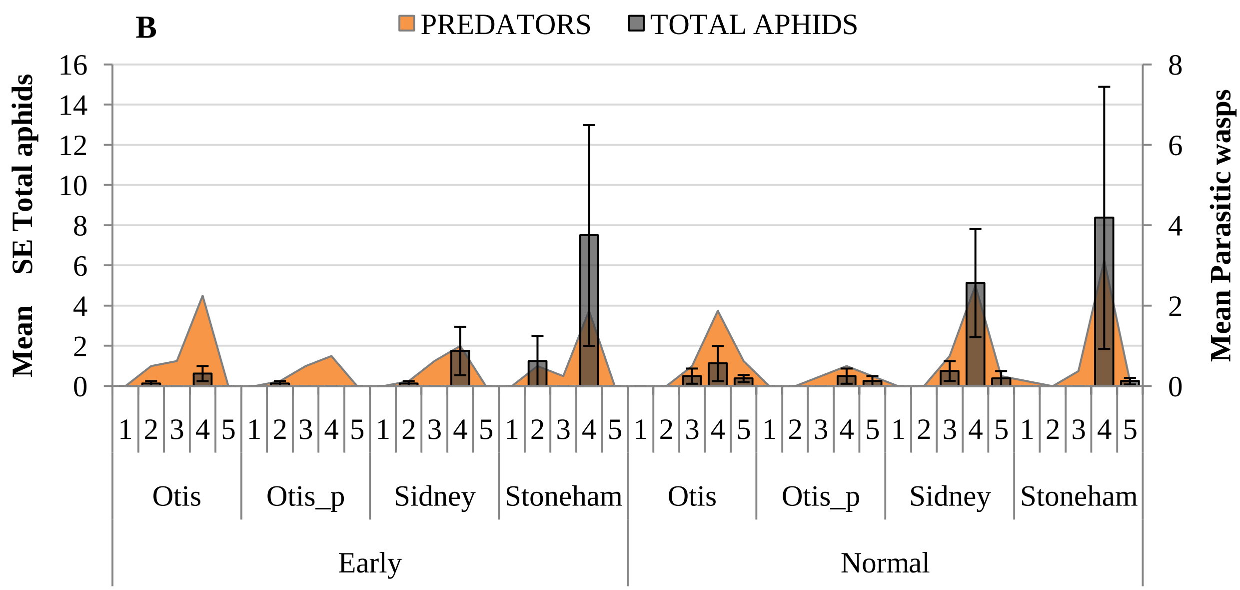
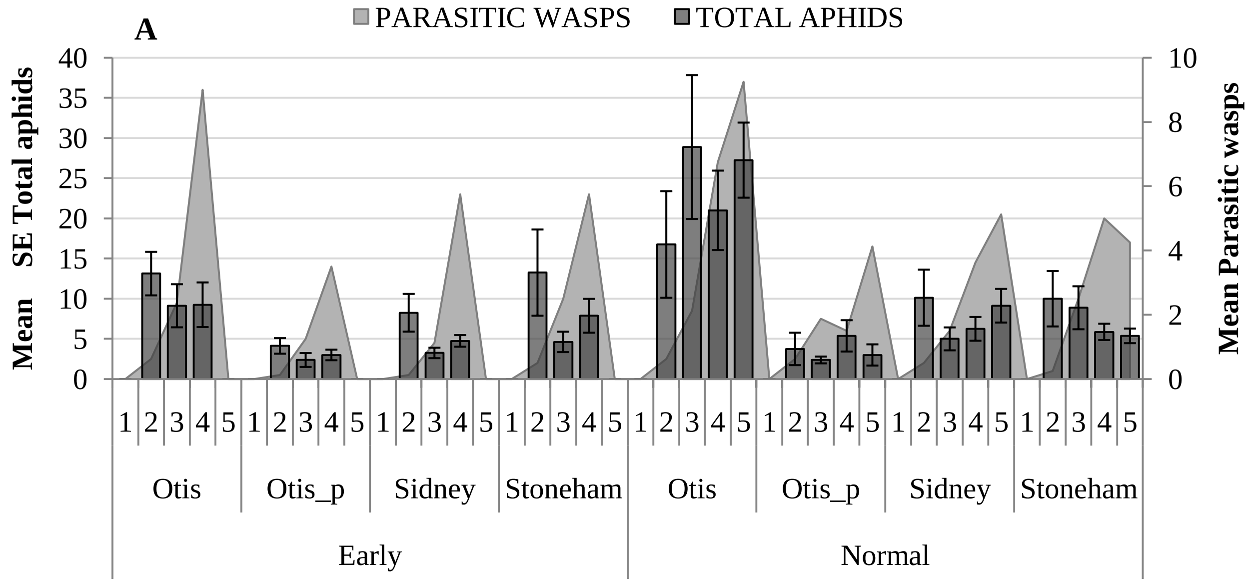


Fig. 4.10 Comparison of mean ± SE Total aphids to mean numbers of A) Parasitic wasps and B) Predators, by planting date, sampling date, and variety, in Nebraska in 2008.

Otis = Susceptible variety, Otis_p = Otis under thiametoxam regime, Resistant varieties = Sidney and Stoneham.



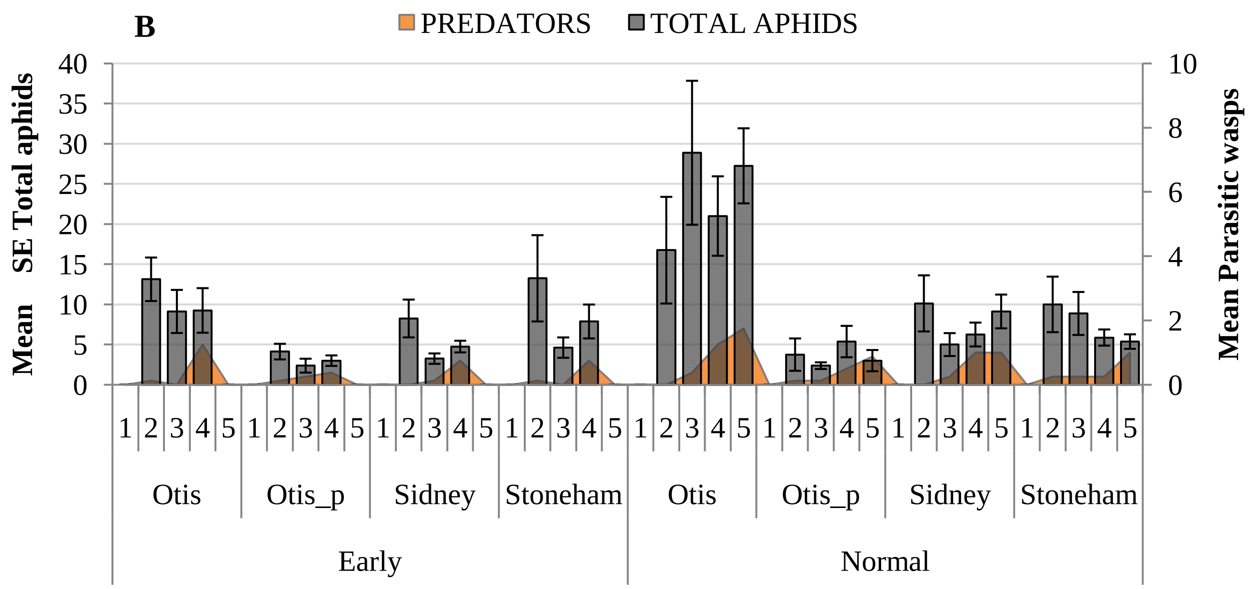
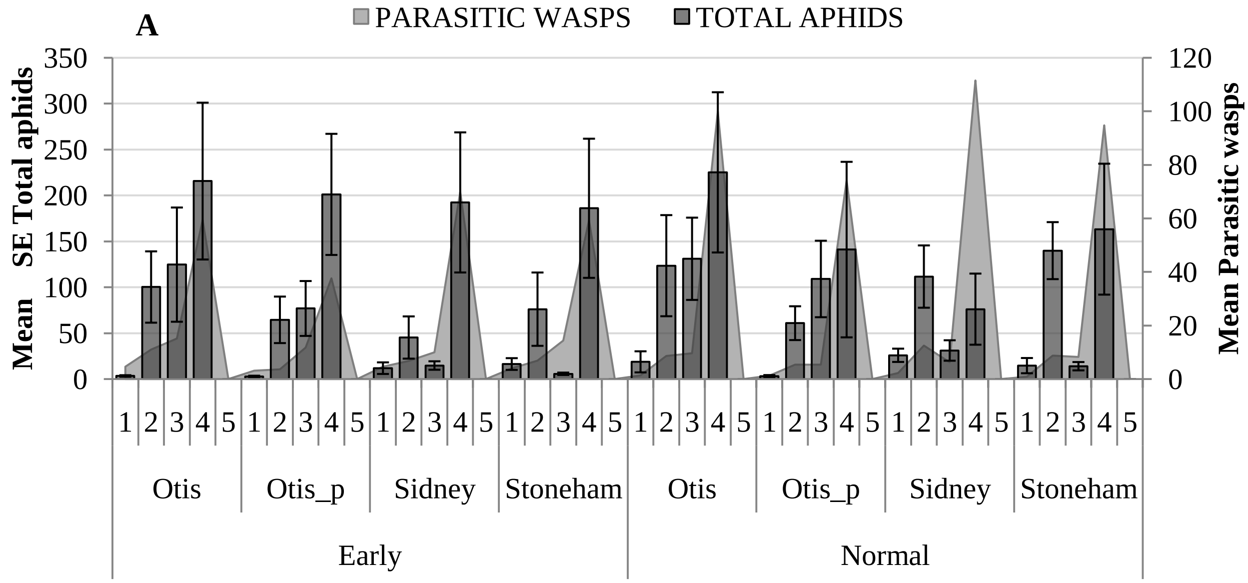


Fig. 4.11 Comparison of mean ± SE Total aphids to mean numbers of A) Parasitic wasps and B) Predators, by planting date, sampling date, and variety, in Colorado in 2009.

Otis = Susceptible variety, Otis_p = Otis under thiametoxam regime, Resistant varieties = Sidney and Stoneham.



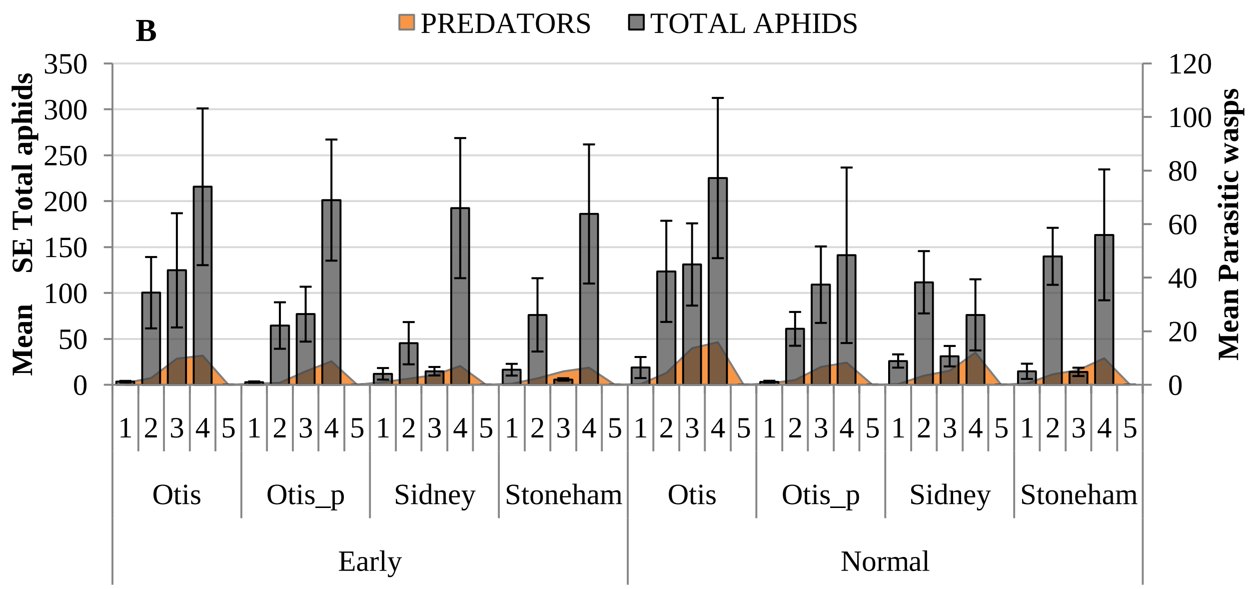
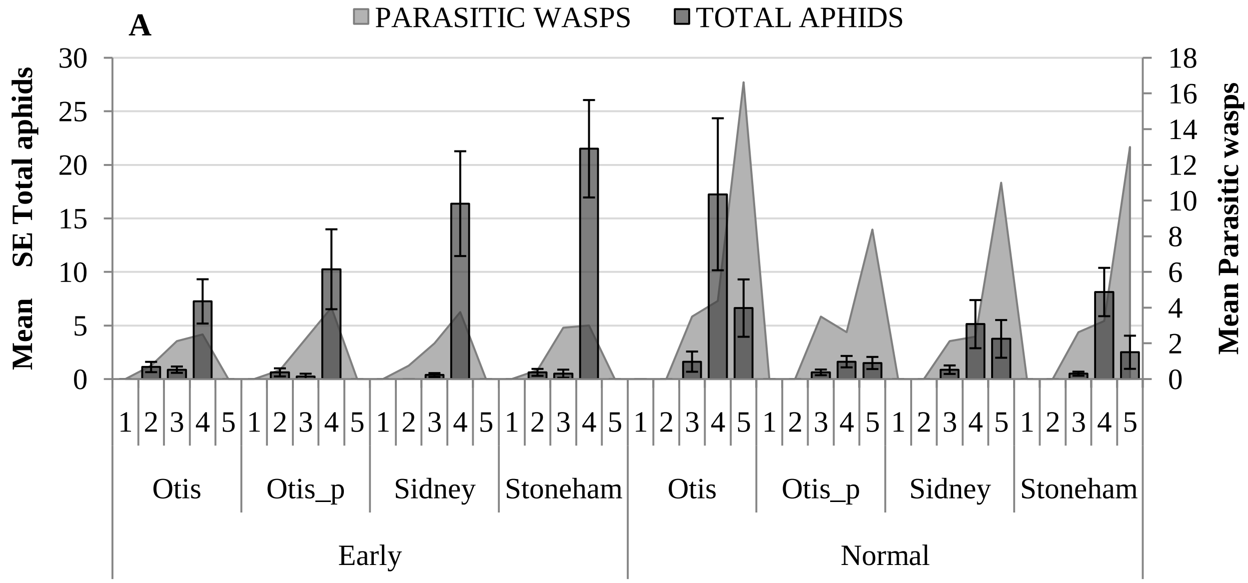


Fig. 4.12 Comparison of mean ± SE Total aphids to mean numbers of A) Parasitic wasps and B) Predators, by planting date, sampling date, and variety, in Kansas in 2009.

Otis = Susceptible variety, Otis_p = Otis under thiametoxam regime, Resistant varieties = Sidney and Stoneham.



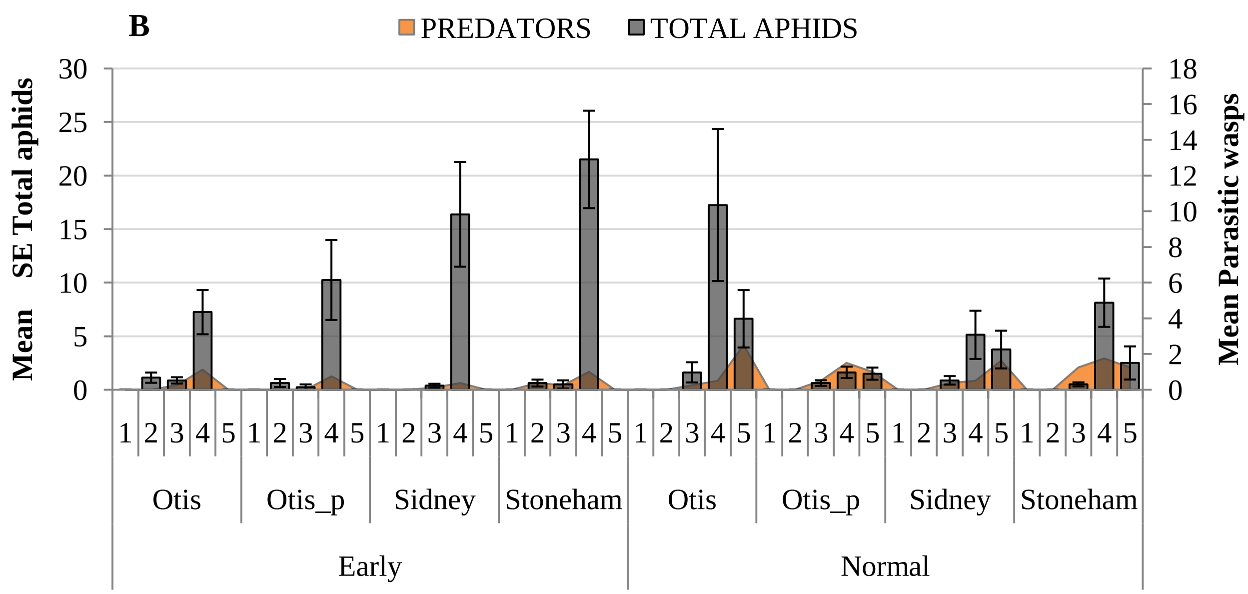


Fig. 4.13 Comparison of mean ± SE Total aphids to mean numbers of A) Parasitic wasps and B) Predators, by planting date, sampling date, and variety, in Nebraska in 2009.

Otis = Susceptible variety, Otis_p = Otis under thiametoxam regime, Resistant varieties = Sidney and Stoneham.

CHAPTER 5 - GENERAL CONCLUSIONS

The objectives of this study were to show how RWA integrated pest management (IPM) strategies affect the presence of other cereal aphid and aphid natural enemy populations in east central Colorado, western Kansas and western Nebraska in 2007, 2008 and 2009. The climate at each geographic location was the most important factor affecting mean RWA population increase, compared to planting date, barley variety or natural enemies. Very dry climatic conditions resulting from high temperatures and moderate levels of precipitation at Tribune, Kansas promoted the highest mean RWA population of any location/year combination in 2009. In contrast, unfavorable conditions resulting from comparably higher levels of precipitation contributed to the lowest RWA populations in all three years of study at Sidney, Nebraska. Moderate climatic conditions at Fort Collins, Colorado intermediate to Tribune and Sidney consistently promoted the development of RWA populations between those at Tribune and Sidney in 2007, 2008 and 2009. These results are similar to those of previous studies (Hughes 1988, Hughes and Maywald 1990) which indicate that very high RWA populations and population outbreaks occur in areas of prolonged drought, such as western Kansas, and that innocuous populations occur in areas of normal or excessive rainfall, such as western Nebraska.

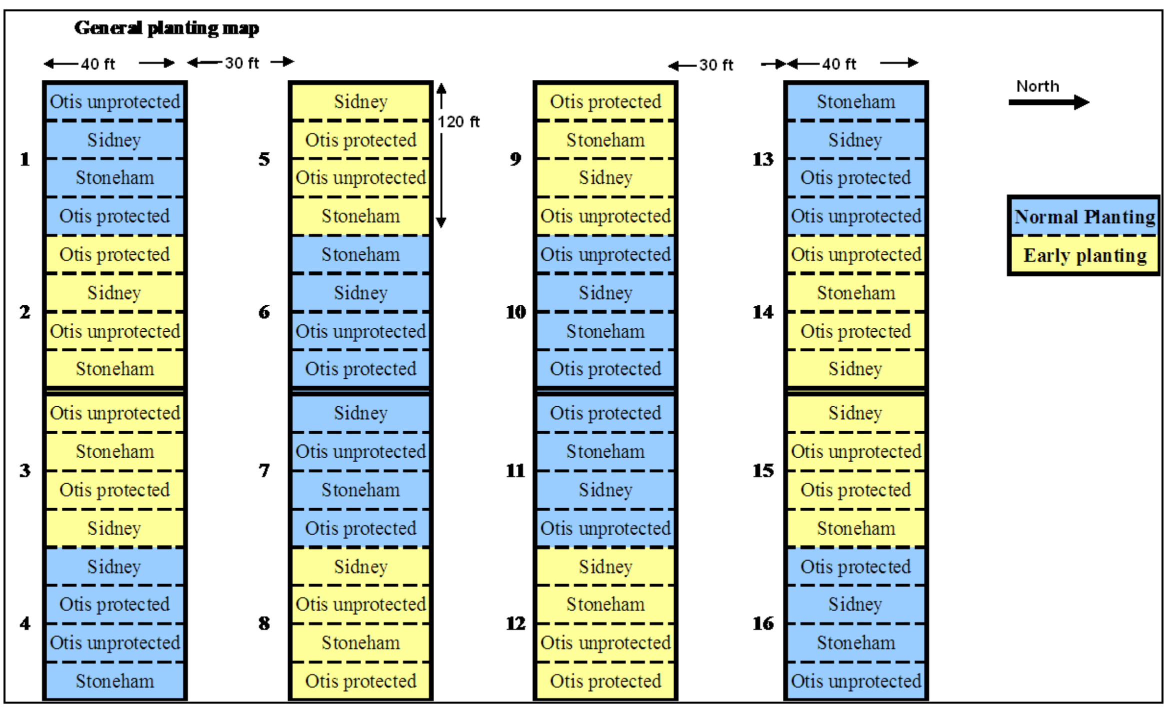
Planting barley one month earlier than normal helped reduce RWA infestations at Fort Collins, Colorado site in all three years of study, and early planting appears promising as a RWA management tactic in western Kansas. No differences in RWA populations were observed on plants in early- and normal planted plots in western Nebraska. A very important result of this research was the determination of an additive effect from early planting date and RWA-resistant barley variety. Early-planted plots of Sidney and Stoneham sustained significantly lower RWA infestations than those occurring on Otis, and were similar to infestations on Otis plants seed-treated with thiamethoxam.

Populations of cereal aphids other than RWA, primarily greenbug and bird cherry oat aphid, were higher at Tribune, Kansas and Fort Collins, Colorado than at Sidney, Nebraska. Early planting and RWA-resistant varieties had no effect on these cereal aphids, and they preferred to feed on resistant varieties, regardless of the level of RWA resistance. As with RWA, populations of other cereal aphids were reduced on Otis plants seed-treated with thiamethoxam.

Conversely, RWA-resistant barley varieties had no negative effects on the abundance of aphid natural enemies. Shannon-Wiener indices and analyses of variance showed no significant differences between the abundance of natural enemies occurring on plants of Sidney, Stoneham, Otis or Otis plants treated with thiamethoxam. Although natural enemies were encountered more frequently in normal planting time plots, no definitive planting date effect was determined.

In general, results of this research indicate that planting RWA-resistant barley varieties approximately one month earlier than normal can significantly reduce RWA infestations in east central Colorado and western Kansas. The overall similarity between RWA populations on the resistant barley varieties and the susceptible cultivar Otis protected by thiamethoxam indicates that RWA- resistant barley varieties should be put into production to reduce RWA-related yield losses. However, the rational use of systemic seed treatments is still recommended to reduce populations of cereal aphid species other than RWA on barley. Finally, the occurrence of aphid natural enemies is not affected by the implementation of RWA-resistant barley varieties or by rational use of systemic insecticides with short residual effects.

Appendix A - Experimental design and general map



Appendix B - SAS Codes for Chapters 2-4

1. The effect of location, planting dates, and varieties was evaluated for seven functional groups: RWA, ‘Other aphids (OtherAp)’, ‘Spiders’, and ‘Parasitoid wasps (PW)’, ‘Coccinellidae beetles (Coccinel)’, ‘Hemiptera predators’, and ‘Neuroptera predators’. The total populations for each functional group were counted across the growing season (4 evaluation dates) in each subplot (varietal treatment). The final average of these counts was obtained by averaging the blocks (8 blocks for each planting date).

```
proc means noprint sum data=ap2;
by Loc block plot PltDate Variety;
     var RWA OtherAp  Spiders PW Coccinel Hemiptera Neuroptera;

output out=sums sum=sumRWA sumOtherAp sumSpider sumPW sumCoccinel
                              sumHemiptera sumNeuroptera;
```

2. Averages for these functional groups were transformed to (log X + 1) in order to reduce the variation of the data and to increase normality in the dispersion of data.

```
data sums; set sums;
  LogRWA=log(sumRWA + 1);
  LogOtherap=log(sumOtherAp + 1);
  LogSpider=log(sumSpider + 1);
  LogPW=log(sumPW + 1);
  LogCoccinel=log(sumCoccinel + 1);
  LogHemiptera=log(sumHemiptera + 1);
  LogNeuroptera=log(sumNeuroptera + 1);
```

3. Transform data was analyzed using PROC MIXED, which is a procedure that accounts for fixed and random effects. The PROC MIXED was done for each functional group, but in this case only PROC MIXED for RWA will be shown.

```
proc mixed covtest Cl data=sums ;
class Loc block Plot Pltdate variety;
model logRWA= Loc|Pltdate|Variety/ddfm=satterth outp=resd;
random block(Loc) Plot(block Loc)
        Pltdate*block(Loc) Pltdate*Plot(block Loc)
        Variety*block(Loc) Variety*Plot(block Loc)
        Variety*Pltdate*block(Loc);
```

4. Orthogonal contrasts were obtained in order to compare the means for the varietal treatments.

```
contrast 'Susceptible vs. Resistant' Variety 2 0 -1 -1;
contrast 'Otis vs. Otis protected' Variety 1 -1 0 0;
contrast 'Otis protected vs. Resistant' Variety 0 2 -1 -1;
```

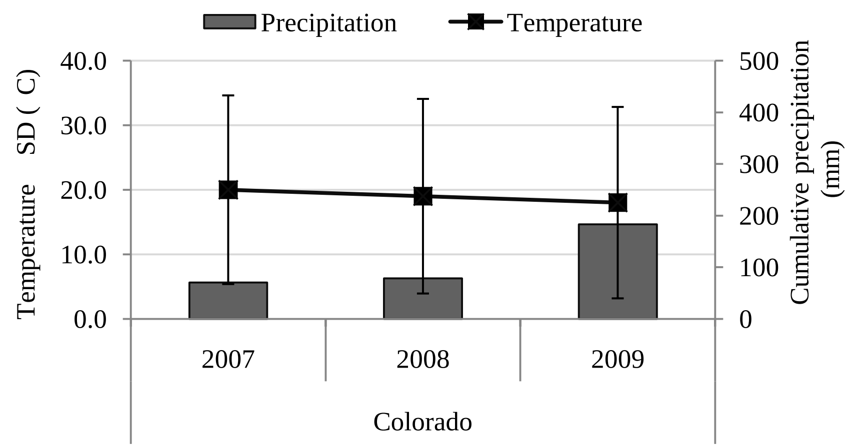
5. Mean significant differences were obtained for locations, varieties, location-varieties, location-planting dates, planting dates-varieties, and location-planting dates-varieties. In order to evaluate the least significant differences within means, pair-wise comparisons were determined by using a t-test and a Bonferroni test.

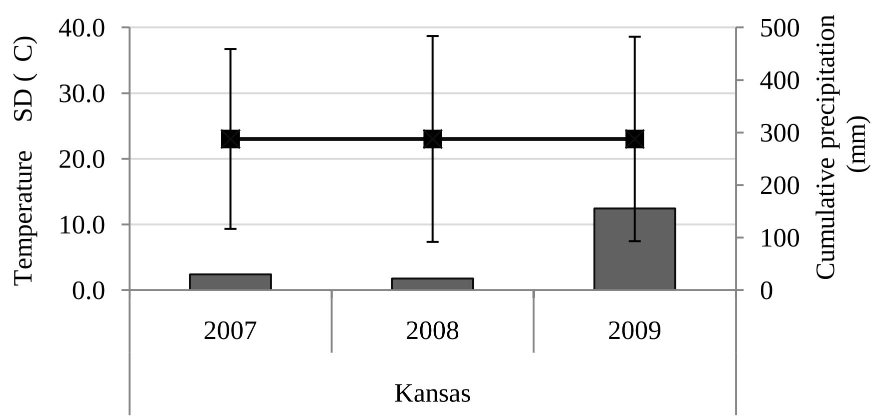
```
lsmeans Loc|Pltdate|Variety/cl;
lsmeans Loc/pdiff adjust=Bon;
lsmeans variety/pdiff adjust=Bon slice=Loc;
lsmeans Loc*Pltdate/pdiff adjust=Bon slice=Loc;
lsmeans Loc*variety/pdiff adjust=Bon slice=Loc;
lsmeans Pltdate*variety/pdiff adjust=Bon;
lsmeans Loc|Pltdate|Variety/pdiff adjust=Bon slice=Loc ;
```

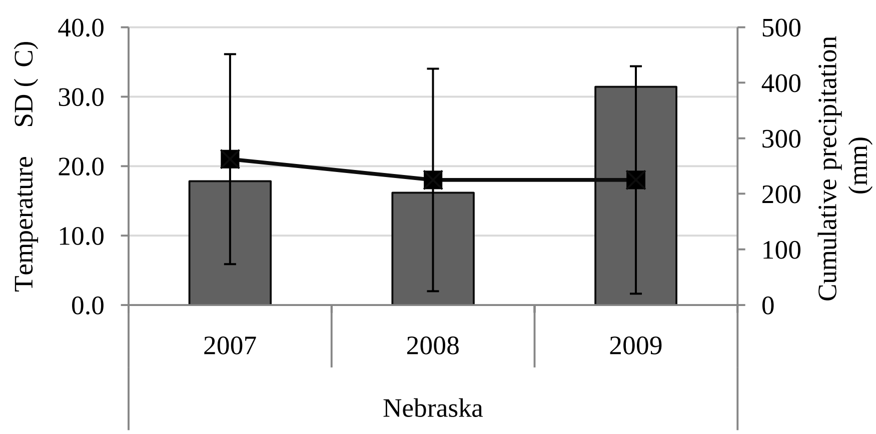
6. A correlation between functional groups was made using PROC CORR procedure. The correlation coefficients were obtained by location-variety

```
proc sort data=sums;
by Loc  variety;
proc corr data=sums;
by Loc variety;
var LogRWA LogOtaphids LogSpider LogTotalPW LogCoccinel LogHemiptera
LogNeuroptera;
```

Appendix C - Chapter 2: Climatic profile (Temperature and precipitation) data for Colorado, Kansas, and Nebraska 2007-2009







Appendix D - Chapter 4: Abundance of natural enemies in Colorado, Kansas, and Nebraska 2007-2009

Table D-1. Abundance of major natural enemy groups on Otis, Sidney and Stoneham barley varieties at Fort Collins, Colorado in 2007

Major groups	Subdivisions	Otis		Otis_p		Sidney		Stoneham	
		n_i	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**
Spiders	Spiders	9	0.11	17	0.32	16	0.22	17	0.26
Parasitic wasps (PW)	Chalcidoidea Superfamily	28	0.35	18	0.34	22	0.30	25	0.38
	Aphelinidae	19	0.24	1	0.02	1	0.01	2	0.03
	Mymaridae	2	0.03	0	0.00	2	0.03	0	0.00
	Braconidae	8	0.10	4	0.08	11	0.15	11	0.17
	Diaeretiella	0	0.00	1	0.02	1	0.01	2	0.03
	Ephedrus	1	0.01	2	0.04	1	0.01	1	0.02
	Praon	0	0.00	0	0.00	0	0.00	0	0.00
	Lysiphlebus	0	0.00	0	0.00	0	0.00	0	0.00
Coccinellidae	*H. convergens*	1	0.01	0	0.00	3	0.04	2	0.03
	H. parenthesis	0	0.00	0	0.00	3	0.04	0	0.00
	C. septempunctata	0	0.00	0	0.00	1	0.01	0	0.00
	C. transversoguttata	0	0.00	0	0.00	0	0.00	0	0.00
	C. trifasciata	0	0.00	0	0.00	0	0.00	0	0.00
	Larvae Coccinellidae	0	0.00	2	0.04	3	0.04	0	0.00
	Hippodamia	0	0.00	0	0.00	0	0.00	0	0.00
	black Coccinellidae	2	0.03	2	0.04	3	0.04	1	0.02
Hemiptera	Nabidae	3	0.04	2	0.04	4	0.05	1	0.02
	Geocoridae	5	0.06	2	0.04	2	0.03	0	0.00
	Anthocoridae	0	0.00	1	0.02	1	0.01	1	0.02
	Pentatomidae	1	0.01	1	0.02	0	0.00	1	0.02
Neuroptera	Chrysopidae	0	0.00	0	0.00	0	0.00	0	0.00
	Hemerobiidae	0	0.00	0	0.00	0	0.00	1	0.02
	Total individuals (N)	**79**	**1.00**	**53**	**1.00**	**74**	**1.00**	**65**	**1.00**
	Taxonomic groups collected (S)	**11**		**12**		**15**		**12**	

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham. **n_i**= Number of individuals collected in each major group by variety; **p_i** = Relative proportion of individuals in each major group with respect with total individuals collected in each variety by location

Table D-2. Abundance of major natural enemy groups on Otis, Sidney and Stoneham barley varieties at Tribune, Kansas in 2007

Major groups	**Subdivisions**	**Otis**		**Otis_p**		**Sidney**		**Stoneham**	
		n_i	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**
Spiders	Spider	13	0.10	14	0.11	18	0.13	16	0.16
Parasitic wasps (PW)	Chalcidoidea Superfamily	54	0.44	52	0.42	79	0.55	34	0.35
	Aphelinidae	6	0.05	2	0.02	3	0.02	1	0.01
	Mymaridae	5	0.04	4	0.03	0	0.00	5	0.05
	Braconidae	12	0.10	21	0.17	11	0.08	13	0.13
	Diaeretiella	4	0.03	1	0.01	0	0.00	0	0.00
	Ephedrus	1	0.01	1	0.01	1	0.01	1	0.01
	Praon	1	0.01	2	0.02	0	0.00	0	0.00
	Lysiphlebus	0	0.00	0	0.00	0	0.00	0	0.00
Coccinellidae	*H. convergens*	2	0.02	3	0.02	3	0.02	1	0.01
	H. parenthesis	1	0.01	0	0.00	0	0.00	0	0.00
	C. septempunctata	0	0.00	1	0.01	0	0.00	2	0.02
	C. transversoguttata	0	0.00	0	0.00	0	0.00	0	0.00
	C. trifasciata	0	0.00	0	0.00	0	0.00	0	0.00
	Larvae Coccinellidae	2	0.02	4	0.03	6	0.04	5	0.05
	Hippodamia	0	0.00	2	0.02	0	0.00	1	0.01
	black Coccinellidae	2	0.02	2	0.02	0	0.00	1	0.01
Hemiptera	Nabidae	6	0.05	7	0.06	7	0.05	5	0.05
	Geocoridae	9	0.07	5	0.04	9	0.06	7	0.07
	Anthocoridae	3	0.02	0	0.00	0	0.00	0	0.00
	Pentatomidae	1	0.01	2	0.02	0	0.00	1	0.01
Neuroptera	Chrysopidae	2	0.02	1	0.01	6	0.04	2	0.02
	Hemerobiidae	0	0.00	0	0.00	1	0.01	2	0.02
	Total individuals (N)	**124**	**1.00**	**124**	**1.00**	**144**	**1.00**	**97**	**1.00**
	Taxonomic groups collected (S)	**17**		**17**		**11**		**16**	

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

n_i= Number of individuals collected in each major group by variety

p_i = Relative proportion of individuals in each major group with respect with total individuals collected in each variety by location

Table D-3. Abundance of major natural enemy groups on Otis, Sidney and Stoneham barley varieties at Sidney, Nebraska in 2007

Major groups	**Subdivisions**	**Otis**		**Otis_p**		**Sidney**		**Stoneham**	
		n_i	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**
Spiders	Spider	13	0.18	12	0.18	24	0.23	11	0.16
Parasitic wasps (PW)	Chalcidoidea Superfamily	24	0.32	26	0.38	33	0.32	24	0.34
	Aphelinidae	5	0.07	4	0.06	8	0.08	3	0.04
	Mymaridae	3	0.04	1	0.01	3	0.03	4	0.06
	Braconidae	4	0.05	3	0.04	3	0.03	0	0.00
	Diaeretiella	0	0.00	0	0.00	0	0.00	0	0.00
	Ephedrus	2	0.03	0	0.00	0	0.00	1	0.01
	Praon	0	0.00	0	0.00	0	0.00	0	0.00
	Lysiphlebus	0	0.00	0	0.00	0	0.00	0	0.00
Coccinellidae	*H. convergens*	1	0.01	1	0.01	0	0.00	0	0.00
	H. parenthesis	0	0.00	0	0.00	0	0.00	0	0.00
	C. septempunctata	0	0.00	0	0.00	2	0.02	0	0.00
	C. transversoguttata	0	0.00	0	0.00	0	0.00	0	0.00
	C. trifasciata	0	0.00	0	0.00	0	0.00	0	0.00
	Larvae Coccinellidae	2	0.03	0	0.00	1	0.01	7	0.10
	Hippodamia	0	0.00	0	0.00	0	0.00	0	0.00
	black Coccinellidae	2	0.03	3	0.04	1	0.01	1	0.01
Hemiptera	Nabidae	4	0.05	4	0.06	6	0.06	4	0.06
	Geocoridae	5	0.07	4	0.06	5	0.05	5	0.07
	Anthocoridae	5	0.07	5	0.07	11	0.11	6	0.09
	Pentatomidae	3	0.04	4	0.06	6	0.06	4	0.06
Neuroptera	Chrysopidae	1	0.01	1	0.01	0	0.00	0	0.00
	Hemerobiidae	0	0.00	0	0.00	0	0.00	0	0.00
	Total individuals (N)	**74**	**1.00**	**68**	**1.00**	**103**	**1.00**	**70**	**1.00**
	Taxonomic groups collected (S)	**14**		**12**		**12**		**11**	

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

n_i= Number of individuals collected in each major group by variety

p_i = Relative proportion of individuals in each major group with respect with total individuals collected in each variety by location

Table D-4. Abundance of major natural enemy groups on Otis, Sidney and Stoneham barley varieties at Fort Collins, Colorado in 2008

Major groups	Subdivisions	Otis		Otis_p		Sidney		Stoneham	
		n_i	p_i	n_i	p_i	n_i	p_i	n_i	p_i
Spiders	Spiders	8	0.07	3	0.05	3	0.03	3	0.03
Parasitic wasps (PW)	Chalcidoidea Superfamily	49	0.43	14	0.25	31	0.32	46	0.42
	Aphelinidae	3	0.03	0	0.00	4	0.04	2	0.02
	Mymaridae	7	0.06	2	0.04	4	0.04	4	0.04
	Braconidae	7	0.06	10	0.18	7	0.07	5	0.05
	Diaeretiella	2	0.02	0	0.00	2	0.02	0	0.00
	Ephedrus	2	0.02	2	0.04	1	0.01	4	0.04
	Praon	0	0.00	0	0.00	0	0.00	0	0.00
	Lysiphlebus	1	0.01	0	0.00	0	0.00	0	0.00
Coccinellidae	*H. convergens*	3	0.03	0	0.00	4	0.04	4	0.04
	H. parenthesis	0	0.00	0	0.00	2	0.02	0	0.00
	C. septempunctata	0	0.00	1	0.02	3	0.03	1	0.01
	C. transversoguttata	1	0.01	0	0.00	0	0.00	0	0.00
	C. trifasciata	1	0.01	1	0.02	0	0.00	4	0.04
	Larvae Coccinellidae	4	0.04	4	0.07	5	0.05	6	0.06
	Hippodamia	0	0.00	0	0.00	0	0.00	0	0.00
	black Coccinellidae	0	0.00	0	0.00	0	0.00	0	0.00
Hemiptera	Nabidae	3	0.03	1	0.02	3	0.03	4	0.04
	Geocoridae	18	0.16	13	0.23	20	0.20	18	0.17
	Anthocoridae	3	0.03	4	0.07	8	0.08	7	0.06
	Pentatomidae	0	0.00	1	0.02	0	0.00	1	0.01
Neuroptera	Chrysopidae	1	0.01	0	0.00	1	0.01	0	0.00
	Hemerobiidae	1	0.01	0	0.00	0	0.00	0	0.00
	Total individuals (N)	**114**		**56**		**98**		**109**	
	Taxonomic groups collected (S)	**17**		**12**		**15**		**14**	

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

n_i= Number of individuals collected in each major group by variety

p_i = Relative proportion of individuals in each major group with respect with total individuals collected in each variety by location

Table D-5. Abundance of major natural enemy groups on Otis, Sidney and Stoneham barley varieties at Tribune, Kansas in 2008

Major groups	**Subdivisions**	**Otis**		**Otis_p**		**Sidney**		**Stoneham**	
		n_i	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**
Spiders	Spider	27	0.10	21	0.14	23	0.12	31	0.09
Parasitic wasps (PW)	Chalcidoidea Superfamily	138	0.52	72	0.47	98	0.52	227	0.67
	Aphelinidae	21	0.08	4	0.03	8	0.04	8	0.02
	Mymaridae	10	0.04	5	0.03	9	0.05	5	0.01
	Braconidae	23	0.09	12	0.08	17	0.09	23	0.07
	Diaeretiella	2	0.01	2	0.01	1	0.01	2	0.01
	Ephedrus	0	0.00	0	0.00	1	0.01	2	0.01
	Praon	0	0.00	0	0.00	0	0.00	0	0.00
	Lysiphlebus	1	0.00	1	0.01	0	0.00	0	0.00
Coccinellidae	*H. convergens*	7	0.03	4	0.03	8	0.04	8	0.02
	H. parenthesis	6	0.02	1	0.01	3	0.02	8	0.02
	C. septempunctata	0	0.00	0	0.00	0	0.00	0	0.00
	C. transversoguttata	0	0.00	0	0.00	0	0.00	0	0.00
	C. trifasciata	0	0.00	0	0.00	0	0.00	0	0.00
	Larvae Coccinellidae	13	0.05	10	0.06	9	0.05	13	0.04
	Hippodamia	4	0.02	0	0.00	3	0.02	2	0.01
	black Coccinellidae	5	0.02	13	0.08	2	0.01	1	0.00
Hemiptera	Nabidae	2	0.01	1	0.01	2	0.01	1	0.00
	Geocoridae	4	0.02	2	0.01	2	0.01	1	0.00
	Anthocoridae	0	0.00	0	0.00	2	0.01	0	0.00
	Pentatomidae	0	0.00	4	0.03	2	0.01	4	0.01
Neuroptera	Chrysopidae	1	0.00	2	0.01	0	0.00	4	0.01
	Hemerobiidae	1	0.00	0	0.00	0	0.00	1	0.00
	Total individuals (N)	**265**		**154**		**190**		**341**	
	Taxonomic groups collected (S)	**16**		**15**		**16**		**17**	

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

n_i= Number of individuals collected in each major group by variety

p_i = Relative proportion of individuals in each major group with respect with total individuals collected in each variety by location

Table D-6. Abundance of major natural enemy groups on Otis, Sidney and Stoneham barley varieties at Sidney, Nebraska in 2008

Major groups	**Subdivisions**	**Otis**		**Otis_p**		**Sidney**		**Stoneham**	
		n_i	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**
Spiders	Spider	10	0.06	6	0.06	5	0.04	5	0.04
Parasitic wasps (PW)	Chalcidoidea Superfamily	72	0.45	50	0.53	68	0.53	60	0.42
	Aphelinidae	3	0.02	4	0.04	5	0.04	5	0.04
	Mymaridae	13	0.08	12	0.13	6	0.05	18	0.13
	Braconidae	14	0.09	10	0.11	8	0.06	8	0.06
	Diaeretiella	0	0.00	0	0.00	0	0.00	2	0.01
	Ephedrus	1	0.01	3	0.03	1	0.01	0	0.00
	Praon	0	0.00	0	0.00	0	0.00	0	0.00
	Lysiphlebus	2	0.01	0	0.00	0	0.00	0	0.00
Coccinellidae	*H. convergens*	5	0.03	1	0.01	2	0.02	1	0.01
	H. parenthesis	2	0.01	0	0.00	1	0.01	1	0.01
	C. septempunctata	0	0.00	0	0.00	0	0.00	0	0.00
	C. transversoguttata	0	0.00	0	0.00	0	0.00	0	0.00
	C. trifasciata	0	0.00	0	0.00	0	0.00	0	0.00
	Larvae Coccinellidae	8	0.05	0	0.00	1	0.01	14	0.10
	Hippodamia	0	0.00	0	0.00	0	0.00	0	0.00
	black Coccinellidae	2	0.01	1	0.01	0	0.00	0	0.00
Hemiptera	Nabidae	6	0.04	1	0.01	1	0.01	1	0.01
	Geocoridae	4	0.03	0	0.00	7	0.05	4	0.03
	Anthocoridae	12	0.08	4	0.04	22	0.17	22	0.15
	Pentatomidae	0	0.00	2	0.02	0	0.00	0	0.00
Neuroptera	Chrysopidae	5	0.03	0	0.00	2	0.02	1	0.01
	Hemerobiidae	0	0.00	0	0.00	0	0.00	0	0.00
	Total individuals (N)	**159**		**94**		**129**		**142**	**1.00**
	Taxonomic groups collected (S)	**15**		**11**		**13**		**13**	

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

n_i= Number of individuals collected in each major group by variety

p_i = Relative proportion of individuals in each major group with respect with total individuals collected in each variety by location

Table D-7. Abundance of major natural enemy groups on Otis, Sidney and Stoneham barley varieties at Fort Collins, Colorado in 2009

Major groups	Subdivisions	Otis		Otis_p		Sidney		Stoneham	
		n_i	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**
Spiders	Spiders	3	0.01	5	0.04	3	0.02	4	0.02
Parasitic wasps (PW)	Chalcidoidea Superfamily	83	0.29	46	0.38	56	0.34	45	0.24
	Aphelinidae	11	0.04	2	0.02	4	0.02	9	0.05
	Mymaridae	15	0.05	7	0.06	14	0.08	14	0.08
	Braconidae	21	0.07	17	0.14	9	0.05	19	0.10
	Diaeretiella	116	0.41	31	0.25	59	0.35	78	0.42
	Ephedrus	0	0.00	0	0.00	0	0.00	0	0.00
	Praon	0	0.00	1	0.01	0	0.00	1	0.01
	Lysiphlebus	0	0.00	0	0.00	0	0.00	0	0.00
Coccinellidae	*H. convergens*	12	0.04	1	0.01	7	0.04	1	0.01
	H. parenthesis	0	0.00	1	0.01	0	0.00	0	0.00
	C. septempunctata	5	0.02	1	0.01	2	0.01	3	0.02
	C. transversoguttata	0	0.00	0	0.00	0	0.00	0	0.00
	C. trifasciata	2	0.01	0	0.00	0	0.00	0	0.00
	Larvae Coccinellidae	5	0.02	0	0.00	5	0.03	0	0.00
	Hippodamia	1	0.00	0	0.00	1	0.01	0	0.00
	black Coccinellidae	1	0.00	0	0.00	0	0.00	0	0.00
Hemiptera	Nabidae	6	0.02	7	0.06	3	0.02	7	0.04
	Geocoridae	0	0.00	1	0.01	0	0.00	0	0.00
	Anthocoridae	0	0.00	1	0.01	0	0.00	3	0.02
	Pentatomidae	0	0.00	0	0.00	0	0.00	0	0.00
Neuroptera	Chrysopidae	2	0.01	1	0.01	4	0.02	2	0.01
	Hemerobiidae	0	0.00	0	0.00	0	0.00	0	0.00
	Total individuals (N)	**283**	**1.00**	**122**	**1.00**	**167**		**186**	
	Taxonomic groups collected (S)	**14**		**14**		**12**		**12**	

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

n_i= Number of individuals collected in each major group by variety

p_i = Relative proportion of individuals in each major group with respect with total individuals collected in each variety by location

Table D-8. Abundance of major natural enemy groups on Otis, Sidney and Stoneham barley varieties at Tribune, Kansas in 2009

Major groups	**Subdivisions**	**Otis**		**Otis_p**		**Sidney**		**Stoneham**	
		n_i	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**
Spiders	Spider	12	0.01	12	0.01	11	0.01	19	0.01
Parasitic wasps	Chalcidoidea Superfamily	1334	0.62	937	0.67	1514	0.73	1351	0.73
(PW)	Aphelinidae	154	0.07	75	0.05	109	0.05	111	0.06
	Mymaridae	77	0.04	46	0.03	74	0.04	44	0.02
	Braconidae	93	0.04	61	0.04	84	0.04	56	0.03
	Diaeretiella	19	0.01	15	0.01	7	0.00	15	0.01
	Ephedrus	0	0.00	4	0.00	1	0.00	0	0.00
	Praon	0	0.00	0	0.00	1	0.00	0	0.00
	Lysiphlebus	0	0.00	0	0.00	0	0.00	0	0.00
Coccinellidae	*H. convergens*	86	0.04	36	0.03	29	0.01	36	0.02
	H. parenthesis	2	0.00	4	0.00	2	0.00	3	0.00
	C. septempunctata	15	0.01	5	0.00	6	0.00	3	0.00
	C. transversoguttata	0	0.00	0	0.00	0	0.00	0	0.00
	C. trifasciata	0	0.00	0	0.00	0	0.00	0	0.00
	Larvae Coccinellidae	175	0.08	94	0.07	96	0.05	78	0.04
	Hippodamia	12	0.01	8	0.01	5	0.00	3	0.00
	black Coccinellidae	5	0.00	1	0.00	3	0.00	2	0.00
Hemiptera	Nabidae	11	0.01	15	0.01	4	0.00	9	0.00
	Geocoridae	7	0.00	2	0.00	2	0.00	1	0.00
	Anthocoridae	95	0.04	61	0.04	94	0.05	88	0.05
	Pentatomidae	1	0.00	1	0.00	0	0.00	0	0.00
Neuroptera	Chrysopidae	36	0.02	12	0.01	18	0.01	25	0.01
	Hemerobiidae	6	0.00	3	0.00	6	0.00	3	0.00
	Total individuals (N)	**2,140**		**1,392**		**2,066**		**1847**	
	Taxonomic groups collected (S)	**18**		**19**		**19**		**17**	

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

n_i= Number of individuals collected in each major group by variety

p_i = Relative proportion of individuals in each major group with respect with total individuals collected in each variety by location

Table D-9. Abundance of major natural enemy groups on Otis, Sidney and Stoneham barley varieties at Sidney, Nebraska in 2009

Major groups	**Subdivisions**	**Otis**		**Otis_p**		**Sidney**		**Stoneham**	
		n_i	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**	**n_i**	**p_i**
Spiders	Spider	7	0.03	6	0.03	6	0.03	6	0.03
Parasitic wasps (PW)	Chalcidoidea Superfamily	137	0.51	109	0.58	120	0.62	112	0.47
	Aphelinidae	24	0.09	8	0.04	11	0.06	7	0.03
	Mymaridae	48	0.18	28	0.15	25	0.13	44	0.19
	Braconidae	24	0.09	22	0.12	17	0.09	28	0.12
	Diaeretiella	6	0.02	3	0.02	3	0.02	10	0.04
	Ephedrus	0	0.00	0	0.00	0	0.00	0	0.00
	Praon	0	0.00	0	0.00	0	0.00	1	0.00
	Lysiphlebus	0	0.00	0	0.00	0	0.00	0	0.00
Coccinellidae	*H. convergens*	1	0.00	1	0.01	0	0.00	2	0.01
	H. parenthesis	0	0.00	1	0.01	0	0.00	1	0.00
	C. septempunctata	1	0.00	0	0.00	0	0.00	3	0.01
	C. transversoguttata	0	0.00	0	0.00	0	0.00	0	0.00
	C. trifasciata	0	0.00	0	0.00	0	0.00	0	0.00
	Larvae Coccinellidae	2	0.01	0	0.00	3	0.02	1	0.00
	Hippodamia	0	0.00	0	0.00	1	0.01	0	0.00
	black Coccinellidae	2	0.01	1	0.01	0	0.00	4	0.02
Hemiptera	Nabidae	2	0.01	2	0.01	1	0.01	1	0.00
	Geocoridae	1	0.00	1	0.01	1	0.01	4	0.02
	Anthocoridae	10	0.04	3	0.02	3	0.02	8	0.03
	Pentatomidae	0	0.00	1	0.01	0	0.00	0	0.00
Neuroptera	Chrysopidae	2	0.01	0	0.00	4	0.02	5	0.02
	Hemerobiidae	0	0.00	1	0.01	0	0.00	0	0.00
	Total individuals (N)	**267**		**187**		**195**		**237**	
	Taxonomic groups collected (S)	**14**		**14**		**12**		**16**	

Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

n_i= Number of individuals collected in each major group by variety

p_i = Relative proportion of individuals in each major group with respect with total individuals collected in each variety by location

Appendix E - Chapter 4: Shannon-Wiener indices, variances, and pair-wise comparisons for each varietal treatment by location during 2007-2009

Table E-1. Shannon-Wiener indices and variances for varietal treatments at Fort Collins, Colorado; Tribune, Kansas and Sidney, Nebraska in 2007, 2008, and 2009

Year	Variety	Colorado		Kansas		Nebraska	
		H index [a]	Variance [b]	H index	Variance	H index	Variance
2007	Otis	0.80	0.0115	0.89	0.0125	0.96	0.0131
	Otis_p	0.80	0.0233	0.87	0.0124	0.87	0.0151
	Sidney	0.93	0.0147	0.70	0.0101	0.87	0.0084
	Stoneham	0.75	0.0183	0.91	0.0123	0.88	0.0108
2008	Otis	0.88	0.0138	0.77	0.0065	0.87	0.0092
	Otis_p	0.89	0.0142	0.81	0.0097	0.70	0.0150
	Sidney	0.95	0.0108	0.78	0.0092	0.71	0.0121
	Stoneham	0.87	0.0122	0.60	0.0065	0.80	0.0080
2009	Otis	0.75	0.0047	0.65	0.0009	0.69	0.0053
	Otis_p	0.78	0.0096	0.60	0.0016	0.63	0.0088
	Sidney	0.76	0.0071	0.50	0.0010	0.61	0.0085
	Stoneham	0.75	0.0060	0.51	0.0012	0.77	0.0066

[a] H index (Shannon-Wiener) calculated for each varietal treatment following the formula: $H' = -\Sigma p_i \ln p_i$. Calculation based on complete p_i values from tables in Appendix D

[b] H index variance calculated for each varietal treatment following the formula:

$$V_{Hw} = \frac{[(\Sigma n_i \log^2 n_i) - (\Sigma n_i \log n_i)^2]/N}{N^2}$$

Table E-2. Pair-wise comparisons between varietal treatments based on Shannon-Wiener indices for natural enemies collected in Fort Collins, Colorado; Tribune, Kansas and Sidney Nebraska in 2007, 2008, and 2009.

Year	**Variety comparisons**	**COLORADO**			**KANSAS**			**NEBRASKA**		
		t-test [a]	**df (v)** [b]	**Sign.**	**t-test**	**df (v)**	**Sign.**	**t-test**	**df (v)**	**Sign.**
2007	Otis vs. Otis_p	**0.02**	2.93E+03	N.S.	**0.36**	9.95E+03	N.S.	**1.24**	4.98E+03	N.S.
	Otis vs. Sidney	**1.84**	5.70E+03	N.S.	**3.05**	1.15E+04	**	**1.43**	7.17E+03	N.S.
	Otis vs. Stoneham	**0.64**	4.36E+03	N.S.	**0.29**	8.80E+03	N.S.	**1.08**	6.01E+03	N.S.
	Otis_p vs. Sidney	**1.51**	2.89E+03	N.S.	**2.68**	1.15E+04	**	**0.01**	5.83E+03	N.S.
	Otis_p vs. Stoneham	**0.56**	2.71E+03	N.S.	**0.65**	8.82E+03	N.S.	**0.25**	5.17E+03	N.S.
	Sidney vs. Stoneham	**2.26**	4.09E+03	*	**3.37**	9.89E+03	**	**0.30**	8.17E+03	N.S.
2008	Otis vs. Otis_p	**0.11**	5.31E+03	N.S.	**0.77**	2.10E+04	N.S.	**2.47**	8.29E+03	**
	Otis vs. Sidney	**1.05**	8.61E+03	N.S.	**0.14**	2.59E+04	N.S.	**2.45**	1.28E+04	**
	Otis vs. Stoneham	**0.19**	8.55E+03	N.S.	**3.45**	4.60E+04	**	**1.23**	1.75E+04	N.S.
	Otis_p vs. Sidney	**0.92**	5.22E+03	N.S.	**0.59**	1.78E+04	N.S.	**0.16**	7.70E+03	N.S.
	Otis_p vs. Stoneham	**0.30**	5.32E+03	N.S.	**3.85**	2.20E+04	**	**1.47**	8.11E+03	N.S.
	Sidney vs. Stoneham	**1.28**	9.00E+03	N.S.	**3.27**	2.74E+04	**	**1.39**	1.27E+04	N.S.
2009	Otis vs. Otis_p	**0.63**	1.71E+04	N.S.	**2.35**	1.12E+06	**	**1.28**	2.71E+04	N.S.
	Otis vs. Sidney	**0.25**	3.13E+04	N.S.	**7.78**	2.10E+06	**	**1.70**	2.89E+04	*
	Otis vs. Stoneham	**0.09**	3.94E+04	N.S.	**7.16**	1.81E+06	**	**1.60**	4.12E+04	N.S.
	Otis_p vs. Sidney	**0.37**	1.58E+04	N.S.	**4.42**	1.11E+06	**	**0.36**	2.20E+04	N.S.
	Otis_p vs. Stoneham	**0.53**	1.64E+04	N.S.	**4.00**	1.06E+06	**	**2.63**	2.58E+04	N.S.
	Sidney vs. Stoneham	**0.16**	2.65E+04	N.S.	**0.35**	1.73E+06	N.S.	**3.04**	2.72E+04	*

[a] t-statistic (t) calculated for each pair-wise comparison between varietal treatments. $T=(Hw_1-Hw_2/Dvar)$

[b] Degree of freedoms, df(v) associated with the value of the t-statistic were calculated for each pair-wise comparisons between varietal treatments. $Df= (var_1 + var_2)^2 / [(Var_1^2/N_1)+ Var_2^2/N_2)]$

** Highly significant, * significant at 5%, N. S. = non significant differences

Appendix F - Chapter 4: Analysis of variance for natural enemies 2007-2009

Table F-1. Analysis of variance for **spiders** populations during 2007-2009 field trips.

Effect	Loc	DF	2007		2008		2009	
			F value	Pr > F	F value	Pr > F	F value	Pr > F
Loc		2	0.10	0.9054	43.81	<.0001	8.43	0.0031
Pltdate	Across locations	1	16.03	0.0021	2.95	0.0884	3.14	0.0956
Loc*Pltdate	Across locations	2	0.58	0.5788	0.02	0.9814	0.08	0.9270
Loc*Pltdate	CO	1	7.39	0.0202	0.74	0.3924	0.98	0.3362
Loc*Pltdate	KS	1	2.06	0.1790	1.28	0.2597	0.58	0.4563
Loc*Pltdate	NE	1	7.73	0.0181	0.97	0.3267	1.72	0.2078
Variety	Across locations	3	1.63	0.1911	0.99	0.4083	0.60	0.6194
Loc*Variety	Across locations	6	0.50	0.8036	0.57	0.7545	0.48	0.8203
Loc*Variety	CO	3	0.55	0.6518	0.40	0.7536	0.11	0.9540
Loc*Variety	KS	3	0.48	0.7000	0.59	0.6268	1.42	0.2543
Loc*Variety	NE	3	1.61	0.1950	1.13	0.3492	0.02	0.9959
Pltdate*variety	Across locations	3	3.98	0.0116	1.50	0.2183	2.22	0.1096
Loc*Pltdate*Variety	Across locations	6	3.67	0.0034	0.40	0.8757	1.62	0.1810
Loc*Pltdate*Variety	CO	7	1.90	0.0885	0.46	0.8571	0.53	0.8087
Loc*Pltdate*Variety	KS	7	3.44	0.0045	1.19	0.3210	1.36	0.2552
Loc*Pltdate*Variety	NE	7	3.10	0.0081	0.67	0.6952	1.59	0.1723

Analysis of variance determined with Proc MIXED, with fixed effects SE method, and degrees of freedom obtained by Satterthwaite method (See Appendix B for SAS Codes)

Total number of observations for the analysis= 192

Number of observations per location= 64, observations per planting date= 96, observations per variety= 48

Number of observations per planting date/location = 32

Number of observations per variety/location = 16

Number of observations per variety/planting date/location = 8

Table F-2. Analysis of variance for **parasitoid wasp (PW)** populations during 2007-2009 field trips.

Effect	Loc	DF	2007		2008		2009	
			F value	Pr > F	F value	Pr > F	F value	Pr > F
Loc		2	2.10	0.1789	13.92	0.0001	131.65	<.0001
Pltdate	Across locations	1	2.45	0.1323	3.29	0.0974	19.08	0.0004
Loc*Pltdate	Across locations	2	6.92	0.0049	2.57	0.1219	3.41	0.0554
Loc*Pltdate	CO	1	15.53	0.0007	0.64	0.4416	1.75	0.2023
Loc*Pltdate	KS	1	0.47	0.4987	4.38	0.0608	2.54	0.1285
Loc*Pltdate	NE	1	0.29	0.5950	3.42	0.0919	21.62	0.0002
Variety	Across locations	3	3.10	0.0329	8.28	0.0004	12.04	<.0001
Loc*Variety	Across locations	6	1.05	0.3996	0.90	0.5106	3.38	0.0067
Loc*Variety	CO	3	2.98	0.0381	5.15	0.0057	13.42	<.0001
Loc*Variety	KS	3	1.10	0.3553	4.57	0.0098	4.74	0.0052
Loc*Variety	NE	3	1.13	0.3436	0.36	0.7838	0.63	0.5993
Pltdate*variety	Across locations	3	0.17	0.9183	0.88	0.4551	0.46	0.7117
Loc*Pltdate*Variety	Across locations	6	0.43	0.8543	0.55	0.7679	1.12	0.3611
Loc*Pltdate*Variety	CO	7	3.54	0.0029	2.47	0.0325	6.02	<.0001
Loc*Pltdate*Variety	KS	7	0.82	0.5757	2.82	0.0171	2.51	0.0264
Loc*Pltdate*Variety	NE	7	0.65	0.7131	1.08	0.3915	4.38	0.0070

Analysis of variance determined with Proc MIXED, with fixed effects SE method, and degrees of freedom obtained by Satterthwaite method (See Appendix B for SAS Codes)

Total number of observations for the analysis= 192

Number of observations per location= 64, observations per planting date= 96, observations per variety= 48

Number of observations per planting date/location = 32

Number of observations per variety/location = 16

Number of observations per variety/planting date/location = 8

Table F-3. Analysis of variance for **Coccinellidae** populations during 2007-2009 field trips.

Effect	Loc	DF	2007		2008		2009	
			F value	Pr > F	F value	Pr > F	F value	Pr > F
Loc		2	2.11	0.1508	5.66	0.0256	75.97	<.0001
Pltdate	Across locations	1	3.80	0.0669	1.35	0.2752	9.60	0.0128
Loc*Pltdate	Across locations	2	3.82	0.0414	0.40	0.6846	1.01	0.4023
Loc*Pltdate	CO	1	2.59	0.1246	1.55	0.2439	0.61	0.4557
Loc*Pltdate	KS	1	7.80	0.0120	0.59	0.4635	7.78	0.0211
Loc*Pltdate	NE	1	1.05	0.3186	0.00	1.0000	3.24	0.1056
Variety	Across locations	3	1.74	0.1690	3.72	0.0153	8.72	<.0001
Loc*Variety	Across locations	6	1.66	0.1498	1.30	0.2686	2.33	0.0359
Loc*Variety	CO	3	4.05	0.0114	1.31	0.2775	5.38	0.0016
Loc*Variety	KS	3	0.79	0.5055	0.56	0.6426	6.73	0.0003
Loc*Variety	NE	3	0.21	0.8866	4.45	0.0065	1.27	0.2869
Pltdate*variety	Across locations	3	0.24	0.8700	1.03	0.3837	0.10	0.9576
Loc*Pltdate*Variety	Across locations	6	0.43	0.8547	1.79	0.1142	0.26	0.9539
Loc*Pltdate*Variety	CO	7	2.19	0.0502	1.67	0.1378	2.50	0.0258
Loc*Pltdate*Variety	KS	7	1.79	0.1095	1.16	0.3444	4.12	0.0010
Loc*Pltdate*Variety	NE	7	0.29	0.9545	2.17	0.0533	1.05	0.4093

Analysis of variance determined with Proc MIXED, with fixed effects SE method, and degrees of freedom obtained by Satterthwaite method (See Appendix B for SAS Codes)

Total number of observations for the analysis= 192

Number of observations per location= 64, observations per planting date= 96, observations per variety= 48

Number of observations per planting date/location = 32

Number of observations per variety/location = 16

Number of observations per variety/planting date/location = 8

Table F-4. Analysis of variance for **hemipteran predators** populations during 2007-2009 field trips.

Effect	Loc	DF	2007		2008		2009	
			F value	**Pr > F**	**F value**	**Pr > F**	**F value**	**Pr > F**
Loc		2	5.01	0.0181	8.14	0.0019	145.82	<.0001
Pltdate	Across locations	1	6.75	0.0187	2.68	0.1173	29.56	<.0001
Loc*Pltdate	Across locations	2	2.02	0.1628	1.70	0.2083	0.12	0.8875
Loc*Pltdate	CO	1	0.83	0.3737	0.27	0.6100	7.51	0.0160
Loc*Pltdate	KS	1	0.22	0.6479	0.01	0.9282	10.96	0.0052
Loc*Pltdate	NE	1	9.74	0.0062	5.80	0.0258	11.34	0.0046
Variety	Across locations	3	0.64	0.5922	2.54	0.0750	3.34	0.0212
Loc*Variety	Across locations	6	0.41	0.8682	0.90	0.5094	0.97	0.4477
Loc*Variety	CO	3	0.32	0.8083	1.05	0.3848	1.32	0.2720
Loc*Variety	KS	3	0.14	0.9379	0.03	0.9932	2.33	0.0770
Loc*Variety	NE	3	1.01	0.3952	3.26	0.0352	1.64	0.1837
Pltdate*variety	Across locations	3	0.45	0.7188	2.93	0.0474	0.05	0.9836
Loc*Pltdate*Variety	Across locations	6	0.46	0.8346	1.08	0.3957	1.85	0.0948
Loc*Pltdate*Variety	CO	7	0.31	0.9474	1.00	0.4448	1.99	0.0686
Loc*Pltdate*Variety	KS	7	0.56	0.7855	0.18	0.9872	3.48	0.0028
Loc*Pltdate*Variety	NE	7	1.89	0.0864	3.72	0.0039	2.66	0.0164

Analysis of variance determined with Proc MIXED, with fixed effects SE method, and degrees of freedom obtained by Satterthwaite method (See Appendix B for SAS Codes)

Total number of observations for the analysis= 192

Number of observations per location= 64, observations per planting date= 96, observations per variety= 48

Number of observations per planting date/location = 32

Number of observations per variety/location = 16

Number of observations per variety/planting date/location = 8

Table F-5. Analysis of variance for **neuropteran predators** populations during 2007-2009 field trips.

Effect	Loc	DF	2007		2008		2009	
			F value	Pr > F	F value	Pr > F	F value	Pr > F
Loc		2	4.35	0.0476	0.64	0.5474	43.85	<.0001
Pltdate	Across locations	1	0.00	0.9597	0.44	0.5095	1.30	0.2614
Loc*Pltdate	Across locations	2	2.00	0.1412	0.13	0.8813	1.49	0.2378
Loc*Pltdate	CO	1	0.27	0.6071	0.20	0.6583	1.27	0.2657
Loc*Pltdate	KS	1	2.68	0.1055	0.49	0.4837	0.53	0.4708
Loc*Pltdate	NE	1	1.07	0.3048	0.00	1.0000	2.47	0.1238
Variety	Across locations	3	0.77	0.5147	1.62	0.2076	2.87	0.0428
Loc*Variety	Across locations	6	1.50	0.1904	1.13	0.3712	1.97	0.0826
Loc*Variety	CO	3	0.16	0.9217	0.43	0.7337	0.20	0.8926
Loc*Variety	KS	3	3.39	0.0223	1.27	0.3048	6.15	0.0009
Loc*Variety	NE	3	0.22	0.8852	2.19	0.1129	0.46	0.7115
Pltdate*variety	Across locations	3	0.24	0.8686	1.74	0.1656	0.64	0.5930
Loc*Pltdate*Variety	Across locations	6	0.64	0.6993	1.32	0.2590	0.52	0.7947
Loc*Pltdate*Variety	CO	7	0.22	0.9794	0.30	0.9521	0.45	0.8668
Loc*Pltdate*Variety	KS	7	2.22	0.0396	1.73	0.1234	3.05	0.0077
Loc*Pltdate*Variety	NE	7	0.40	0.9019	1.61	0.1532	0.74	0.6368

Analysis of variance determined with Proc MIXED, with fixed effects SE method, and degrees of freedom obtained by Satterthwaite method (See Appendix B for SAS Codes)

Total number of observations for the analysis= 192

Number of observations per location= 64, observations per planting date= 96, observations per variety= 48

Number of observations per planting date/location = 32

Number of observations per variety/location = 16

Number of observations per variety/planting date/location = 8

Appendix G - Chapter 4: Correlations between RWA and other cereal aphids with major groups of natural enemies in Colorado, Kansas, and Nebraska 2007-2009

Table G-1. Colorado, 2007

Functional group	Otis [a]		Otis_p		Sidney		Stoneham	
	RWA [b]	**Other aphids**	**RWA**	**Other aphids**	**RWA**	**Other aphids**	**RWA**	**Other aphids**
Other aphids	-0.21	1.00	0.00	1.00	-0.09	1.00	-0.41	1.00
	0.4370		0.9860		0.7517		0.1152	
Spider	0.32	-0.42	0.30	0.26	0.33	-0.43	0.12	-0.42
	0.2226	0.1042	0.2656	0.3372	0.2126	0.0926	0.6554	0.1010
PW	**0.49**	**-0.57**	0.15	0.17	**0.58**	-0.28	0.35	-0.41
	0.0527	**0.0210**	0.5838	0.5350	**0.0180**	0.3023	0.1786	0.1151
Coccinellidae	0.32	-0.27	0.13	0.08	0.11	0.18	0.32	0.03
	0.2213	0.3164	0.6341	0.7595	0.6790	0.5052	0.2272	0.9143
Hemiptera	0.38	-0.74	0.19	-0.21	0.43	0.17	0.14	0.13
	0.1503	0.0011	0.4861	0.4444	0.1003	0.5236	0.6052	0.6293
Neuroptera	.	.	.	.	.	.	0.17	0.03
	.	.	.	.	.	.	0.5409	0.9009

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Correlation value is presented at the top, and the probability value for that correlation is at the bottom. The coefficient values were obtained with the Pearson Correlation Coefficients, N = 16. Bold values are significant at 5%

Table G-2. Kansas, 2007

Functional group	Otis [a]		Otis_p		Sidney		Stoneham	
	RWA [b]	**Other aphids**	**RWA**	**Other aphids**	**RWA**	**Other aphids**	**RWA**	**Other aphids**
Other aphids	0.14	1.00	**-0.51**	1.00	0.08	1.00	-0.03	1.00
	0.6170		**0.0446**		0.7641		0.9048	
Spider	-0.32	0.36	0.38	-0.39	0.22	-0.03	0.38	-0.39
	0.2307	0.1705	0.1409	0.1346	0.4088	0.9151	0.1437	0.1326
PW	-0.22	0.29	0.28	**-0.55**	0.42	0.38	0.15	-0.32
	0.4205	0.2692	0.2957	**0.0267**	0.1068	0.1503	0.5911	0.2344
Coccinellidae	0.22	0.10	0.11	-0.41	-0.08	-0.18	0.32	-0.06
	0.4201	0.7253	0.6785	0.1167	0.7695	0.5054	0.2302	0.8268
Hemiptera	-0.05	-0.03	0.41	-0.42	-0.19	0.28	0.30	-0.09
	0.8649	0.9234	0.1161	0.1034	0.4707	0.2872	0.2525	0.7334
Neuroptera	-0.40	-0.11	-0.02	-0.33	-0.13	-0.08	**0.50**	0.02
	0.1219	0.6976	0.9379	0.2147	0.6358	0.7777	**0.0512**	0.9441

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Correlation value is presented at the top, and the probability value for that correlation is at the bottom. The coefficient values were obtained with the Pearson Correlation Coefficients, N = 16. Bold values are significant at 5%

Table G-3. Nebraska, 2007

Functional group	Otis [a] RWA [b]	Otis [a] Other aphids	Otis_p RWA	Otis_p Other aphids	Sidney RWA	Sidney Other aphids	Stoneham RWA	Stoneham Other aphids
Other aphids	0.31	1.00	-0.22	1.00	-0.02	1.00	0.10	1.00
	0.2390		0.4087		0.9480		0.7216	
Spider	-0.44	-0.39	**0.53**	-0.06	-0.14	**-0.55**	-0.16	-0.28
	0.0888	0.1336	**0.0365**	0.8350	0.6127	**0.0287**	0.5604	0.2881
PW	0.24	0.06	0.32	0.11	-0.23	-0.17	-0.01	0.27
	0.3740	0.8303	0.2300	0.6739	0.3932	0.5348	0.9578	0.3204
Coccinellidae	-0.30	**-0.57**	0.03	-0.01	0.20	-0.25	-0.11	-0.24
	0.2528	**0.0206**	0.9119	0.9656	0.4575	0.3597	0.6837	0.3651
Hemiptera	0.30	0.00	**0.61**	0.01	-0.13	-0.28	0.17	**-0.64**
	0.2668	0.9977	**0.0126**	0.9836	0.6336	0.2855	0.5321	**0.0078**
Neuroptera	**-0.64**	**-0.53**	0.44	-0.19	.	.	.	.
	0.0073	**0.0359**	0.0864	0.4852	.	.	.	.

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Correlation value is presented at the top, and the probability value for that correlation is at the bottom. The coefficient values were obtained with the Pearson Correlation Coefficients, N = 16. Bold values are significant at 5%

Table G-4. Colorado, 2008

Functional group	Otis [a]		Otis_p		Sidney		Stoneham	
	RWA [b]	Other aphids	RWA	Other aphids	RWA	Other aphids	RWA	Other aphids
Other aphids	**0.57**	1.00	-0.25	1.00	0.35	1.00	0.27	1.00
	0.0207		0.3561		0.1830		0.3187	
Spider	0.21	0.05	0.20	0.16	-0.04	-0.31	-0.14	-0.17
	0.4395	0.8622	0.4605	0.5578	0.8848	0.2431	0.5939	0.5313
PW	0.04	0.03	-0.37	-0.13	-0.06	0.11	0.10	-0.11
	0.8888	0.9224	0.1529	0.6283	0.8349	0.6820	0.7028	0.6932
Coccinellidae	-0.06	**0.50**	-0.01	0.31	0.23	0.40	-0.05	-0.24
	0.8158	**0.0502**	0.9804	0.2394	0.3884	0.1198	0.8631	0.3714
Hemiptera	-0.34	-0.27	0.28	0.00	0.00	0.24	-0.05	-0.37
	0.2038	0.3184	0.2952	0.9950	0.9907	0.3796	0.8512	0.1635
Neuroptera	0.21	0.03	-0.32	-0.32	.	.	.	.
	0.4259	0.8982	0.2262	0.2227	.	.	.	.

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Correlation value is presented at the top, and the probability value for that correlation is at the bottom. The coefficient values were obtained with the Pearson Correlation Coefficients, N = 16. Bold values are significant at 5%

Table G-5. Kansas, 2008

Functional group	Otis [a]		Otis_p		Sidney		Stoneham	
	RWA [b]	**Other aphids**	**RWA**	**Other aphids**	**RWA**	**Other aphids**	**RWA**	**Other aphids**
Other aphids	**0.51**	1.00	0.17	1.00	0.39	1.00	0.00	1.00
	0.0423		0.5286		0.1399		0.9958	
Spider	0.19	**0.60**	-0.03	-0.18	-0.16	-0.12	0.08	-0.47
	0.4889	**0.0139**	0.9026	0.5002	0.5469	0.6559	0.7696	0.0689
PW	-0.06	-0.43	0.27	0.25	0.08	-0.31	-0.26	**0.48**
	0.8319	0.0958	0.3143	0.3539	0.7721	0.2499	0.3282	**0.0590**
Coccinellidae	-0.11	0.27	**0.56**	0.31	0.30	0.17	-0.43	0.20
	0.6842	0.3091	**0.0256**	0.2398	0.2602	0.5287	0.0968	0.4550
Hemiptera	0.13	0.07	-0.18	0.26	0.03	0.03	0.46	-0.20
	0.6267	0.7870	0.4933	0.3290	0.9006	0.8986	0.0766	0.4525
Neuroptera	0.05	0.20	-0.05	0.13	.	.	-0.17	**-0.54**
	0.8673	0.4576	0.8420	0.6385	.	.	0.5290	**0.0316**

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Correlation value is presented at the top, and the probability value for that correlation is at the bottom. The coefficient values were obtained with the Pearson Correlation Coefficients, N = 16. Bold values are significant at 5%

Table G-6. Nebraska, 2008

Functional group	Otis [a]		Otis_p		Sidney		Stoneham	
	RWA [b]	Other aphids	RWA	Other aphids	RWA	Other aphids	RWA	Other aphids
Other aphids	-0.12	1.00	.	1.00	-0.30	1.00	0.09	1.00
	0.6464		.		0.2507		0.7427	
Spider	-0.10	0.12	.	-0.30	-0.14	0.15	**0.67**	0.00
	0.7192	0.6609	.	0.2596	0.5935	0.5680	**0.0045**	0.9865
PW	-0.07	0.20	.	-0.24	0.00	0.43	-0.32	0.01
	0.8040	0.4652	.	0.3668	0.9964	0.0943	0.2342	0.9729
Coccinellidae	-0.19	0.16	.	-0.22	-0.12	-0.18	-0.26	-0.35
	0.4907	0.5487	.	0.4125	0.6576	0.5071	0.3402	0.1876
Hemiptera	-0.46	**0.56**	.	-0.34	0.20	**0.64**	0.29	-0.11
	0.0744	**0.0226**	.	0.1916	0.4527	**0.0074**	0.2805	0.6944
Neuroptera	-0.10	0.32	.	0.25	-0.07	-0.30	-0.13	0.11
	0.7192	0.2227	.	0.3513	0.8062	0.2507	0.6192	0.6818

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Correlation value is presented at the top, and the probability value for that correlation is at the bottom. The coefficient values were obtained with the Pearson Correlation Coefficients, N = 16. Bold values are significant at 5%

Table G-7. Colorado, 2009

Functional group	Otis [a]		Otis_p		Sidney		Stoneham	
	RWA [b]	Other aphids	RWA	Other aphids	RWA	Other aphids	RWA	Other aphids
Other aphids	0.13	1.00	-0.12	1.00	-0.01	1.00	0.41	1.00
	0.6397		0.6554		0.9659		0.1160	
Spider	0.39	-0.11	-0.05	-0.29	0.03	-0.12	0.19	0.08
	0.1358	0.6747	0.8638	0.2708	0.9010	0.6530	0.4893	0.7775
PW	0.22	0.20	0.19	-0.36	0.03	0.03	0.44	0.36
	0.4165	0.4521	0.4824	0.1721	0.9142	0.9175	0.0865	0.1681
Coccinellidae	0.13	0.40	-0.07	0.27	-0.24	-0.32	0.06	-0.18
	0.6203	0.1245	0.7852	0.3111	0.3776	0.2339	0.8177	0.5095
Hemiptera	0.26	0.25	0.35	0.18	0.06	-0.03	-0.11	-0.28
	0.3324	0.3489	0.1901	0.5000	0.8238	0.9209	0.6827	0.2981
Neuroptera	0.09	0.44	0.46	-0.08	0.34	**0.49**	0.41	0.08
	0.7331	0.0897	0.0751	0.7809	0.1946	**0.0566**	0.1114	0.7729

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Correlation value is presented at the top, and the probability value for that correlation is at the bottom. The coefficient values were obtained with the Pearson Correlation Coefficients, N = 16. Bold values are significant at 5%

Table G-8. Kansas, 2009

Functional group	Otis [a]		Otis_p		Sidney		Stoneham	
	RWA [b]	Other aphids	RWA	Other aphids	RWA	Other aphids	RWA	Other aphids
Other aphids	**0.83**	1.00	0.32	1.00	**0.76**	1.00	**0.65**	1.00
	<.0001		0.2302		**0.0006**		**0.0068**	
Spider	0.34	0.05	0.05	0.16	-0.02	-0.06	0.13	-0.18
	0.1968	0.8528	0.8666	0.5476	0.9506	0.8310	0.6202	0.4941
PW	-0.04	-0.07	0.05	-0.24	0.31	**0.59**	-0.09	-0.21
	0.8848	0.7874	0.8439	0.3718	0.2436	**0.0166**	0.7425	0.4282
Coccinellidae	0.33	0.05	-0.02	-0.03	0.26	0.42	-0.28	-0.26
	0.2113	0.8615	0.9328	0.8986	0.3229	0.1074	0.2878	0.3222
Hemiptera	0.16	0.22	0.08	-0.19	0.11	0.18	-0.45	-0.40
	0.5626	0.4026	0.7589	0.4713	0.6802	0.5140	0.0835	0.1240
Neuroptera	0.05	0.19	0.35	0.33	-0.10	0.05	-0.14	-0.07
	0.8509	0.4807	0.1796	0.2112	0.7169	0.8410	0.6107	0.7865

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Correlation value is presented at the top, and the probability value for that correlation is at the bottom. The coefficient values were obtained with the Pearson Correlation Coefficients, N = 16. Bold values are significant at 5%

Table G-9. Nebraska, 2009

Functional group	Otis [a]		Otis_p		Sidney		Stoneham	
	RWA [b]	Other aphids	RWA	Other aphids	RWA	Other aphids	RWA	Other aphids
Other aphids	**0.59**	1.00	-0.21	1.00	-0.35	1.00	-0.30	1.00
	0.0157		0.4257		0.1871		0.2614	
Spider	0.04	0.39	0.12	-0.19	0.11	-0.05	0.23	-0.10
	0.8723	0.1393	0.6663	0.4798	0.6778	0.8682	0.3818	0.7263
PW	0.48	0.40	0.19	-0.39	0.24	**-0.50**	-0.36	-0.15
	0.0592	0.1261	0.4809	0.1369	0.3626	**0.0469**	0.1658	0.5763
Coccinellidae	0.02	0.36	0.26	-0.40	0.21	**-0.56**	-0.03	-0.27
	0.9322	0.1769	0.3279	0.1248	0.4284	**0.0237**	0.9026	0.3126
Hemiptera	-0.08	0.32	0.21	-0.29	0.03	-0.12	0.29	-0.16
	0.7753	0.2216	0.4315	0.2835	0.9204	0.6702	0.2808	0.5652
Neuroptera	0.27	0.16	-0.33	0.03	0.44	-0.26	-0.20	-0.26
	0.3092	0.5583	0.2152	0.8990	0.0917	0.3299	0.4666	0.3364

[a] Otis = Susceptible variety, Otis_p = Otis treated with thiametoxam, Resistant varieties = Sidney and Stoneham.

[b] Correlation value is presented at the top, and the probability value for that correlation is at the bottom. The coefficient values were obtained with the Pearson Correlation Coefficients, N = 16. Bold values are significant at 5%