MECHANISMS FOR REPRODUCTIVE ISOLATION IN TWO CONGENERIC PARASITOIDS OF THE WHEAT STEM SAWFLY.

by

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April 2013

DEDICATION

This Master's Thesis is for the people of Montana. I hope the findings of this study will contribute to the development of an effective IPM strategy for the wheat stem sawfly and better understanding of its natural enemies.

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ABSTRACT

Cephus cinctus Norton, the wheat stem sawfly, is Montana's most damaging wheat pest. The species is responsible for large yield reductions across the northern Great Plains, costing hundreds of millions of dollars every year. Two congeneric braconid parasitoid species, *Bracon cephi* Gahan and *Bracon lissogaster* Muesebeck (Hymenoptera: Braconidae), are found simultaneously in Montana wheat fields, are active at the same time of year and both use *C. cinctus* as a host. Their role as biological control agents of *C. cinctus* is currently being explored. It is unknown how these morphologically similar parasitoid species maintain reproductive isolation. This study explored several mechanisms allowing *B. cephi* and *B. lissogaster* to remain reproductively isolated and exposed new areas of study and questions of interest regarding the reproductive isolation of these species. No differences in reproductive timing were observed using field-based population abundance surveys, suggesting that alternative isolation mechanisms are being used. A group of candidate sex pheromones analyzed for presence, absence, and relative concentration in each of the parasitoid species' Dufour's glands indicated substantial differences between the two species. These differences suggest a possible role for the Dufour's gland in maintaining reproductive isolation. However, these candidate sex pheromones did not produce significantly different electrophysiological responses in *B. cephi* and *B. lissogaster*. Although this suggests that these candidate sex pheromones may not play a role, mating trials and behavioral assays conducted to assess the interactions between sex and species indicated that the species maintain reproductive isolation in laboratory settings.

INTRODUCTION

The wheat stem sawfly (*Cephus cinctus* Norton) is the most significant pest of wheat in the northern Great Plains region of North America. Originally observed in native grasses in Colorado (Norton 1872, Davis et al. 1955), the wheat stem sawfly quickly made the transition to wheat, followed the westward expansion of intensive agriculture across the American prairies and has been found in wheat fields across the high plains of Montana, Wyoming, Nebraska, Saskatchewan, Alberta and Manitoba ever since (Fletcher 1896, Ainslie 1920). Enjoying a recent resurgence in these areas (Morrill et al. 1998, Meers 2005, Beres et al. 2007), the wheat stem sawfly now constitutes one of the greatest agricultural challenges faced in recent memory by wheat growers and scientists alike.

While the exact economic impact of this pest is difficult to calculate, its effects can be estimated. Montana wheat growers produced 174,970,000 bushels of wheat in 2011 (USDA National Agricultural Statistics Service 2011). Estimated annual losses of $100 million dollars per year (MSU Wheat Stem Sawfly Lab unpublished data; grower estimates) due to wheat stem sawfly equates to approximately 13% of the value of the State of Montana's 2011 wheat harvest (USDA National Agricultural Statistics Service 2011).

Caught off-guard by the resurgence of the wheat stem sawfly in the mid 1990's in Montana (Morrill et al. 1998), researchers and others across the northern Great Plains are now aware of the extent of the wheat stem sawfly infestation in their own states and provinces, forging a necessary cooperation in the painstaking development of an integrated pest management (IPM) strategy that will address the latest outbreak in the 100

year history of this wheat pest. As part of this strategy, entomologists at Montana State University are investigating how natural enemies target and kill *C. cinctus* as part of ongoing efforts to develop the necessary biological control agents. As part of these efforts, the biology and behavior of naturally occurring populations of two endemic parasitoids of the wheat stem sawfly, *Bracon cephi* Gahan and *Bracon lissogaster* Muesebeck (Hymeoptera: Braconidae), are being studied.

Similar in their habits, method of parasitism, and general appearance, these two species also exhibit spatial and temporal synchrony in wheat in fields in north-central Montana. This lack of temporal and spatial separation, while sharing the same host, raises questions about the nature of their interactions. As a result, this study aims to elucidate some of the mechanisms through which concurrently active sympatric populations of *B. cephi* and *B. lissogaster* maintain reproductive isolation. The first part of this study attempted to confirm whether or not there are concurrent populations of the parasitoids *B. cephi* and *B. lissogaster* in Montana wheat fields during the summer and to what extent the populations of *B. cephi* and *B. lissogaster* temporally overlap. A difference in the timing of reproduction is one way these two species could be maintaining their isolation.

The second part of this study examined potential forms of chemical communication responsible for the maintenance of reproductive isolation: sex pheromones are widely used by insects to maintain species isolation, and several acetate esters (*n*-tetradecyl acetate, (*Z*)-7-hexadecen-1-yl acetate, (*Z*)-9-hexadecen-1-yl acetate, and (*Z*)-11-hexadecen-1-yl acetate) identified in the Dufour's glands of *B. cephi* and *B. lissogaster* (Baker et al. 2005) may elicit different physiological and behavioral activities

in the two species. The amounts and ratios of these potential sex pheromones in the Dufour's glands of these two parasitoids were investigated. Finally, live insect mating and behavioral trials examining the response of individual parasitoids to both sexes of conspecifics and of the congeneric species were used to characterize interactions between the two species.

Statement of Problem

Currently, it is unknown how these two endemic parasitoids of the wheat stem sawfly maintain reproductive isolation in wheat field environments across their natural range in the northern Great Plains. The relative ease with which gradual evolutionary shifts in the pheromone composition of populations of insects can occur (Symonds and Elgar 2007) suggests a potential form of reproductive isolation for these two species. Understanding mechanisms of reproductive isolation of these two species is crucial to developing a potential chemical ecology component of an integrated pest management strategy for *C. cinctus*.

Purpose and Rationale

The purpose of this study was to examine the role that a series of endogenous acetate esters may play in maintaining reproductive isolation between these two parasitoid species, as well as to characterize the reproductive behavior of these two species. By first investigating the spatial and temporal concurrence of these two species through field abundance surveys, the necessary rationale was established to explore alternative methods of reproductive isolation. Because Baker et al. (2005) identified a

certain series of acetate esters as having a variety of reproductive attributes in a number of insect orders and families and has been observed in the Dufour's glands of *B. cephi* and *B. lissogaster*, their analysis is an informed starting point for further investigations into the chemical ecology of these two species. Finally, understanding the behavioral interactions of these two species potentially contributes to a more practical characterization of their reproductive habits.

LITERATURE REVIEW

Life History and Biology of *Cephus cinctus* Norton

Montana's most important perennial pest of wheat, *Triticum aestivum* L., is the wheat stem sawfly (*Cephus cinctus* Norton). Discovered in native grasses at the end of the 19th century in Colorado (Norton 1872, Davis et al. 1955), the wheat stem sawfly has been reducing wheat yields since the inception of wheat farming on the high plains of Montana, Wyoming, Nebraska, Saskatchewan, Alberta, and Manitoba (Ainslie 1920).

Shortly after its discovery, the wheat stem sawfly became established in wheat agroecosystems since the late 19th century, causing the greatest yield losses in wheat in Alberta, Manitoba, Saskatchewan (Canada) and Montana, Wyoming, and North and South Dakota (USA) (Wallace and McNeal 1966, Weiss and Morrill, 1992). Throughout the last 50 years, the wheat stem sawfly has exhibited sporadic resurgences across its range, but these have been temporary and somewhat geographically isolated (Holmes 1977 and 1982, Morrill 1983). In the mid 1990's, Montana experienced a major increase in populations of the wheat stem sawfly (Morrill et al. 1998), shortly followed by dramatic increases in the neighboring Canadian provinces of Alberta and Saskatchewan (Meers 2005, Beres et al. 2007). Currently, the wheat stem sawfly is found in 29 states in the western U.S. and in the southern portions of 7 Canadian provinces (Ainslie 1920, Ivie 2001, Morrill 1997), with the greatest damage occurring in Montana, Alberta, and Saskatchewan.

Taxonomically, *Cephus cinctus* is placed in the family Cephidae, suborder Symphyta, order Hymenoptera, class Insecta and the phylum Arthropoda. Traditionally considered endemic to North America, the wheat stem sawfly has been proposed as a recently invasive species (Ivie and Sinojev 1996, Ivie 2001) from Asia, though the mechanism of introduction and the identification of the synonymous Asian population have not been confirmed.

The unique life history of the wheat stem sawfly plays an especially important role in shaping the traits which make management this wheat pest difficult, and has contributed to the evolution of specific behaviors and traits of the braconid parasitoids which use it as a host. Understanding the life history of the wheat stem sawfly informs our knowledge of the biology of *B. cephi* and *B. lissogaster,* and the potential mechanisms by which these two parasitoids have maintained reproductive isolation.

Adult Wheat Stem Sawflies:

The adult wheat stem sawfly is approximately 8-13 mm length with an elongated, laterally compressed black body with three characteristic lateral yellow stripes across the abdomen. The wings are a smoky gray, while legs are yellow from the coxa to the tarsus. Wheat stem sawflies are sexually dimorphic, with females substantially larger than males and possessing a long, dark, saw-like ovipositor (Fletcher 1904).

Adults are poor fliers and undertake brief flights between locations, typically nearby plants. Most of their flight patterns seem to be reproductive in function, often manifesting as a peculiar hovering flight into the wind. When found on wheat stems, their

bodies are usually oriented head down with their legs closely aligned to the axis of their body (Ainslie 1920, Criddle 1922).

Wheat stem sawflies are univoltine and all females of the species are diploid, possessing 18 chromosomes, while the males are haploid, possessing only nine chromosomes. Thus, unmated females lay eggs which only produce male progeny, while mated females may produce female progeny via selective fertilization (Holmes 1979). Adult lives span between five and eight days, depending on the temperature, humidity, wind conditions and availability of water. The timing of the adult emergence is driven by temperature (Perez-Mendoza and Weaver 2006), with emergence occurring from May through July, depending on local environmental conditions. Adult males are first to emerge, ensuring that once the population of females emerges there is an already established population of reproductive males waiting for them. The majority of females that emerge early are more likely to successfully mate, and most eggs they deposit give rise to female progeny. Female adults that emerge later are more likely to remain virgins and lay mostly male-producing eggs (Holmes 1979).

Oviposition by Wheat Stem Sawflies:

There are a variety of environmental factors which affect wheat stem sawfly oviposition. Temperature, humidity, ambient and incident light levels, wind, and the condition of the host all play a role in shaping the timing and location of oviposition. Females prefer succulent, large stems from which the spike has not yet emerged (boot stage) (Holmes and Peterson 1960), with larger stem diameters producing sex ratios of offspring that are female-biased , and smaller diameter stems favoring sex ratios of

offspring that are male-biased (Wall 1952, Morrill et al. 2000, Carcamo et al. 2005). Females typically produce between 33 and 50 eggs, but generally lay only one egg per stem (Ainslie 1920, Holmes 1979). Multiple eggs per wheat stem are laid in fields with high levels of infestation due to sequential oviposition events by different females (Criddle 1923, Perez-Mendoza et al. 2006, Buteler et al. 2009), with no clear evidence of host-avoidance behavior elicited by changes in plant chemicals after oviposition (Nansen et al. 2005b, Buteler et al. 2009).

The stage of growth of the host wheat plant is an important factor that determines the location and timing of wheat stem sawfly egg deposition (Holmes and Peterson, 1960). Females are known to generally prefer to oviposit through the softer regions of the internodal tissue whenever possible. Early in wheat plant growth, females exhibit preferences for stems with greater elongation of the internodes, while later in plant growth, when the wheat stem has become more rigid and mature females exhibit a preference for the less mature "tillers" on the same plant. Females usually deposit eggs in the second to last developing internode, which is located higher on the stem. Stem diameter also plays an important role in determining the placement of eggs, with females preferring to oviposit in stems with a greater outside diameter (Ainslie 1920, Morrill et al. 1992, 2000). Buteler et al. (2010) showed that when hosts are abundant, females can exhibit highly selective host preferences; this selectivity disappears when hosts are sparse and under laboratory conditions this can result in females attempting to oviposit in glass rods, wooden dowels, or dry wheat stems (Holmes and Peterson 1960).

Ainslie (1920) observed the oviposition behavior of the wheat stem sawfly and provided an excellent brief description of this activity:

> "After selecting the suitable stem for oviposition, the female will walk to the top of the stem in a characteristic way, carefully surveying the stem. She then slowly descends in an antennal-exploring fashion with the antennae held horizontally in front of the head occasionally touching the surface of the stem. After selecting a suitable oviposition site, with the head in a downward position she slowly arches the abdomen and grasps the stem with her hind pair of legs introducing her saw-like ovipositor into the outer tissue of the stem. This insertion process is repeated several times often with a twisting movement as if trying to make a larger opening. Finally the ovipositor is completely inserted into the stem and the sawfly remains motionless for about half a minute, probably depositing the egg in the stem. After the egg is deposited the ovipositor is withdrawn and the incision in the stem closes. The female then flies away in search for another plant where to oviposit."

Wheat Stem Sawfly Eggs:

Oviposition places the egg on the inside wall of the wheat stem or within a hollow space created by the action of the saw-like ovipositor. The egg is milky-white and crescent shaped. Egg sizes are positively correlated with female body size. Egg sizes range from 1 to 1.25 mm long and from 0.33 to 0.42 mm wide (Ainslie 1920). There is a seven-day incubation period, after which the larva hatches from the egg and begins its consumption of the interior surface tissues of the wheat stem (Ainslie 1920).

Wheat Stem Sawfly Larvae:

Though larvae are initially colorless and translucent (Criddle 1923, Ainslie 1929), their diet begins to lend them a greenish-yellow color after feeding begins, complimented by a light brown head capsule and accented with dark four-denticled mandibles. Lacking legs, the larvae use their bristle-equipped caudal horn to aid in propulsion inside of the

stem (Wallace and McNeal, 1966). Mature larval size ranges from 8 to 14 mm in length and 1 to 2 mm in diameter.

The feeding activity of the larva is characterized by its consumption of the vascular and parenchymous tissues directly above and below the position where the egg was laid, forming distinct "galleries" within the internodal segments. Larvae tend to migrate upwards, increasing the size of their galleries and consuming other larvae they may encounter in the process. This usually results in only one larva reaching maturity per wheat stem (Criddle 1923, Seamans et al. 1944; Holmes 1982).

The spatial distribution of larvae in wheat fields is driven by the emergence and oviposition of the adult females; in a traditional one-year fallow strip rotation, females emerging from the previous year's stubble fly upwind toward the edge of the current year's planting, ovipositing in the wheat stems encountered along the edge of the field. As the season progresses, more eggs are deposited toward the center of the field, resulting in a fairly uniform distribution of eggs. However, the distribution of larvae remains clustered around the field edges, perhaps the result of eggs in the field interiors being laid late in the season and not affording the larvae enough time to prepare for overwintering before the senescence of the host begins (Nansen et al. 2005b).

Between late July and early September, as the wheat on the northern Great Plains matures and begins to rapidly desiccate, larvae in the upper chambers of the hollowed-out wheat stem begin to migrate downward towards the base of the plant. It has been proposed that the increased transmission of infrared light through desiccated plant cells in the wall of the wheat plant triggers this response in the larvae (Holmes 1979, 1982).

Upon reaching the base of the wheat stem at ground level, the larvae prepare to enter diapause for the duration of the winter months. The larvae cut a horizontal "v-shaped" groove around most of the circumference of their wheat stems, making the stem easily dislodged from its base at the slightest provocation once the plant is dry and brittle (Runyon et al. 2002).

Directly beneath this cut, at or below ground level, the larvae form hibernaculum chambers where they position their transparent cocoons, plugging the entrances to these chambers (directly beneath the v-shaped groove) with frass and other plant material to survive the cold winter months (Ainslie 1920). The larval stage can be as brief as 30 days before entrance to diapause, but diapause must continue for at least 90 days of exposure to 10 °C before the next emergence. The breaking of diapause and the emergence of a new instar is triggered by warming temperatures in the spring. In late May, larvae pupate. There are five instars for the wheat stem sawfly (Farstad 1940, Holmes 1978).

Wheat Stem Sawfly Pupae:

Wheat stem sawfly pupae are exarate, with free appendages, and an average length of 12 mm and width of 1.5 mm. Initially pale white, the legs and body slowly darken over a period of several days until black. During this 7 to 14 day period, the pupae are initially motionless, but begin to move after several days inside the overwintering chambers (Ainslie 1920). At the end of the pupal stage, the pupa metamorphoses and emerges from its hibernaculum by pushing out the plug of frass from its overwintering chamber and digging through any soil or debris which may be covering the cut stem.

Wheat Stem Sawfly Host Plants

The wheat stem sawfly originally inhabited native grasslands in the northern Great Plains, feeding in the stems of large native and introduced grasses (Ainslie 1920, Criddle 1922) such as *Agropyron* spp., *Lolium* spp., timothy grass (*Phleum pratense* L.), and smooth brome grass (*Bromus inermis* L.). Populations have recently been observed exhibiting strong ovipositional preference for the ubiquitous, introduced *Bromus tectorum* L. (downy brome, or "cheatgrass) grass found throughout North America as well (Perez-Mendoza et al. 2006). The prescient warning of C.V. Riley and C.L. Marlatt in 1891 of the wheat stem sawfly migrating into cereal grain fields (cited in Ainslie 1920) was proven correct because in 1895 James Fletcher reported *C. cinctus* damage to wheat crops in Manitoba and Saskatchewan (cited in Ainslie 1920).

Since that initial discovery, damage to grain crops across the northern Great Plains has increased as areas of land originally given over to native grasslands were plowed under and cultivated with large-stem cereal grains (Farstad 1940, Davis 1955, Holmes 1978). Since the initial discovery in wheat fields in 1891 and today, the wheat stem sawfly has infested nearly all types of cultivated grains and wild grasses found on the northern Great Plains. Oat, however, remain resistant to infestation, reportedly due to a lack of an essential nutrient needed for *C. cinctus* development (Farstad 1940).

Crop Damage Caused by Wheat Stem Sawflies

Wheat stem sawflies consistently cause the loss of millions of bushels of wheat and millions of dollars of revenue every year in the northern Great Plains states and

provinces in the USA and Canada (Davis 1955, Weiss and Morrill 1992). It can be found in such high concentrations in some regions that almost every stem in a field is cut (Ainslie 1929). Yield losses due to infestation are caused both by the physical cutting of the wheat stem by the larvae at pre-diapause, increasing the input costs associated with harvesting grain from the lodged material (Beres 2007), as well as by the reduced head weight caused from the physiological injury to the plant resulting from loss of vascular and parenchyma tissues (Holmes 1954, 1977, Morrill and Kushnak 1996).

Photosynthetic rates in uninfested wheat plants can be 12% higher than in infested plants (Macedo et al. 2007), likely the result of the consumption of vascular and parenchymous tissues by the larvae. Yield losses of 5-35% result from mining, which reduces both the grain weight and the number of kernels per head (Seamans et al. 1938, Munro 1947, McNeal et al. 1955, Wallace and McNeal 1966, Morrill et al. 1994, Delaney et al. 2010).

Chemical Control of Wheat Stem Sawflies

Chemical control methods have been exhaustively researched in the past 50 years in an attempt to find a suitable insecticide to limit the spread of the wheat stem sawfly and reduce its damage. Munro (1949) tested several insecticides (DDT, chlordane, toxaphene, parathion, BHC, and DDD) over a period of two years in wheat fields and concluded that none of the chemicals produced a satisfactory method of control. Granular heptachlor applied in a furrow with wheat seed was reported by Wallace (1962) to cause a 73% reduction in *C. cinctus* numbers, but it was later determined that a reduction of this

degree could only be accomplished when the infestation was minimal and restricted to the lower internodes of the wheat stems. Additionally, it was determined that the rates at which heptachlor was effective in causing a significant reduction in larval numbers resulted in residues in the wheat straw—at higher rates, trace amounts were also observed in grain (Wallace and Butler 1967). Heptachlor has been banned for commercial sale in the United States since 1988 because of soil persistence and has been recovered in crops 15 years after its application (Anonymous 1999), making it unsuitable for wheat stem sawfly management.

Lorsban (chlorpyrifos), Furadan (carbofuran), Warrior (lambda-cyhalothrin), Gaucho 75W (imidacloprid), and ethyl parathion have all been found to be ineffective in controlling populations of wheat stem sawflies (Blodgett et al. 1996, Gall and Dogger 1967; Holmes 1978, Anonymous 1997). It is thought that since the wheat stem sawfly spends the majority of its larval stage inside of the wheat plant, the walls of the wheat stem provide an effective barrier against insecticides and most means of chemical control (Munro et al. 1949, Holmes and Peterson 1963, Holmes 1978). The most vulnerable stage, when it is unprotected by the stem walls of the wheat plant, is during emergence and flight, which occur over a period of several weeks. However, the difficulties in predicting this emergence and the cost of multiple applications of foliar insecticide to cover the entire period makes chemical control infeasible for the wheat stem sawfly (Knodel et al. 2009). Additionally, the potential negative effects of general applications of foliar insecticides on non-target beneficial insect species make this approach to sawfly management less desirable.

Cultivation Practices for Wheat Stem Sawfly Management

Tillage:

Because most larvae form their hibernaculum at or below ground level inside the wheat stem, the temperature inside the stem is moderated during the winter by the insulating properties of soil and snow, protecting the larvae against extreme desiccation and changes in temperature. Tillage can expose the stubble in which hibernating individuals are living in an attempt to kill the larvae through freezing and desiccation (Callenbach and Hansmeier 1944). Desiccation and freezing of infested wheat stems are both considered important contributors to mortality (Salt 1961), resulting in their potential use as an effective control method (Holmes and Farstad 1956).

Tilling in the fall has produced 90% mortality in North Dakota (Weiss et al, 1987) and Holmes and Farstad (1956) reported 99% mortality through fall tillage and between 98 and 100% mortality through the use of spring tillage. The reported mortality rates due to fall and spring tillage ranges from 25% to 100% (McBride et al. 1989, Holmes 197, Callenbach and Hansmeier 1944), indicating that the timing of the tillage, as well as the amount of exposure that the infested stubs will have to the cold, dry winter air seems to play an important role in determining the success or failure of this method of control (Salt 1946, Holmes and Farstad 1956, Morrill et al. 1993). For wheat stem sawflies to be winter-killed, they must be exposed to temperatures of -20 °C for more than 10 days (Carcamo and Beres 2006). In most cases, this can only be accomplished if the infested wheat stub and crown is uprooted and exposed at ground level during exceptionally cold

conditions. Alternatively, plowing infested stubble under to a depth of 15 cm or more one autumn, then completely burying all stubble the following autumn has been suggested as a successful way to reduce populations by increasing the amount of soil the emerging adults with have to dig through and simultaneously exposing the overwintering larvae to increased moisture and fungal/bacterial pathogens (Criddle 1922b).

However, the improper use of this practice also leads to a reduction in parasitoid populations which tend to overwinter higher in wheat stems than *C. cinctus* does and are equally, if not more, vulnerable to the same mortality effects as sawflies under the same treatment. Because naturally occurring populations of parasitoids potentially offer a long-term way to control *C. cinctus* populations, tillage can be detrimental to the development of a multifaceted approach to managing this pest (Morrill et al. 1998, Runyon et al. 2002, and Beres et al. 2011b). In addition, the amount and depth of tillage necessary to achieve high overwintering mortality rates is a serious contributor to soil erosion. The lack of standing residue reduces snow trapping and moisture infiltration into the soil during the winter and spring. A recent study conducted in southern Alberta indicates that a direct-seeding system consisting of a pre-seed heavy-tine harrow followed by a 30-cm knife opener equipped air drill, when used to re-crop wheat fields instead of letting them lie fallow, reduced emergence in the spring by 50-70% without requiring heavy tillage (Beres et al. 2011). Combined with other control measures, re-cropping may be an effective management component.

Plant Spacing and Density:

Wheat plots with wider row spacing (33 cm) experienced greater yield losses than comparable wheat plots with narrower row spacing (7.62-15.24 cm), and rows with a higher plant density often have lower cutting rates than rows with lower plant densities (Lunginbill 1958). There also seems to be a relationship between the degree of infestation and the density of the wheat stem, with solid-stem wheat varieties being more resistant to infestation (Farstad 1940). However, this is largely contingent upon the amount of sunlight received by individual plants, meaning that seeding rates for solid-stem varieties must often be adjusted to maximize pith formation. Recent evidence indicates that resistance is a function of both wheat cultivar and seeding rate, with solid and hollow stem varieties requiring different seeding densities to minimize cutting and infestation. Beres et al. (2011) found that the solid-stem cultivar Lillian grown in Alberta had optimized grain yield and stable pith at seeding densities of 250 and 350 seeds m^{-2}, while higher sowing densities for hollow-stem wheat of 350 to 450 seeds m^{-2} resulted in lowered rates of infestation and optimized grain yield. Wider row spacing and decreased plant densities allow more light to penetrate the wheat field canopy, which can lead to greater pith expression in solid-stemmed varieties as well as an increase in drought tolerance and water soluble carbohydrates (Saint Pierre et al. 2010)

Crop Rotation:

Continuous monoculture wheat plantings in the same field, year after year, tend to produce ideal conditions for *C. cinctus* populations to grow (Callenbach and Hansmeier 1945, Butcher 1946). McBride (1996) found that rotating plantings of wheat with

plantings of more resistant crops such as oat (*Avena sativa* L.), barley (*Hordeum vulgare* L.), soybean (*Glycine max* L.), sunflower (*Helianthus annus* L.), and other legumes (*Fabaceae*) reduced overall populations. A rotation of canola (*Brassica napus* L. (Brassicaceae)), wheat, and field pea (*Pisum sativum* L. (Fabaceae)) provided optimum yield for all three rotations in Saskatchewan (Brandt et al. 2008). However, limitations of crop rotation practices are economic, the concerns of which are often the most important drivers of planting decisions for grain producers, resulting in crop rotation not receiving full utilization by growers
(Weiss and Morrill 1992).

Trap Crops:

Capitalizing on the wheat stem sawfly's preference for certain varieties of wheat with large diameter and hollow stems, trap strips are planted around the edges of a wheat field with the intent to focus the sawfly infestation into crops which are more attractive but which will be destroyed after infestation (Morrill et al. 1992, 2001a). Although this method has proven successful in reducing infestation rates in the interior of wheat fields planted with trap strips (Morrill and Kushnak 1996; Morrill et al. 2001a), many growers are reluctant to destroy the wheat planted in the trap strips due to the considerable investment in planting and maintaining a trap strip (Goosey 1999). In addition, trap strips only seem to be effective at low and moderate levels of infestation, and even then infestation rates as low as 10-15% can lead to rates as high as 80% the following year if the larvae in the trap crop are allowed to enter diapause (Farstad et al. 1945, Holmes 1982).

Delayed Planting:

Delayed planting of wheat crops has received considerable attention as a way to reduce populations. Delayed planting works because stem elongation occurs after most eggs are laid (Jacobsen and Farstad 1952); as a result, delayed planting is recommended for fields with heavy infestations (Morrill and Kushnak 1999). However, delayed planting means that there is less soil moisture available during plant development, resulting in decreased crop yields (Morrill and Kushnak 1996, 1999).

Inherent Plant Resistance:

Farstad (1940) discovered that stem solidness was correlated with increased *C. cinctus* resistance in certain varieties of wheat. The amount and consistency of the pith in the lumen had a direct effect on survival rates of *C. cinctus,* resulting in the creation of the first solid-stem wheat variety, "Rescue". Rescue was a hard red spring wheat designed to be resistant and demonstrated decreased rates of infestation (Holmes and Peterson 1962, Luginbill and Knipling 1969).

Subsequent studies demonstrated that weather patterns had an effect on wheat stem solidness, with cool, wet and cloudy weather producing more hollow stems, and dry, clear and hot weather producing more solid stems with higher resistance (Roemhild 1954). Since the introduction of "Rescue", a number of solid-stem varieties of wheat have been bred for resistance. Cutless, Glenman, Leader, Lew, Tioga, Fortuna, and Lancer are some of the better known varieties (McBride, 1996), as well as Choteau (Lanning et al. 2004) but the increased resistance of these varieties is offset by their decreased yields,

resulting in limited acceptance by growers (Weiss and Morrill 1992). Carcamo et al. (2005) showed that large diameter hollow-stem wheat varieties increased *C. cinctus* fitness, whereas solid-stemmed varieties decreased adult female size and fecundity, and Beres et al. (2007) showed that in environments with moderate to high wheat stem sawfly pressure, solid-stemmed varieties could produce comparable or superior yields and grain protein levels to hollow-stemmed varieties.

In certain situations, blending cultivars (planting two varieties together in one field in a homogenous block) with complementary strengths (Bowden et al. 2001) may improve the overall grain quality in the field (Beres et al. 2007), as well as increase yield under low to moderate levels of *C. cinctus* pressure, but not necessarily under high pressure (Weiss et al. 1990). Beres et al. (2009) reported that a 1:1 blend of solid-stemmed 'AC-Eatonia' and hollow-stemmed 'AC-Barrie' in Alberta resulted in an 11% increase in yield potential when compared to a monoculture of 'AC-Barrie'.

The general health and nutrient balance of the wheat plant may also play a role in inherent resistance. Applications of nitrogen and phosphorus have been reported to actually increase rates of stem cutting (Luginbill and McNeal 1954), though this could be due to the subsequent increase in the wheat plant stem diameter resulting in greater oviposition preference, while a recent greenhouse study indicated that phosphorus deficient plants were more vulnerable to *C. cinctus* damage (Delaney et al. 2010). The diverse variability of soil composition and nutrient levels across the wheat growing areas of the northern plains suggests that differences in plant available nutrients may influence the location of wheat stem sawfly hotspots, but more research is needed.

Biological Control of Wheat Stem Sawflies

The effectiveness of biological control of wheat stem sawflies is a contentious issue, with wide variability in mortality rates of *C. cinctus* reported from a number of studies (Somsen and Luginbill 1957, Smith 1959, Beirn 1972, Morrill et al. 1998; Runyon et al. 2002). However, successful suppression of *C. cinctus* infestations has been reported (Streams and Coles 1965, Morrill et al. 1994, 1998). Soil fungal pathogens (*Fusarium*) can infect larvae, with several species (*F. graminearum, F. acuminatum, F. equiseti,* and *F. avenaceum*) known to be lethal to larvae. There has been discussion about exploiting these fungal pathogens for biological control of *C. cinctus* (Wenda-Piesik et al. 2009), but members of this species complex cause crown rot diseases in wheat.

Phyllobaenus dubius (Wolcott) (Coleoptera: Cleridae) a predatory beetle, has been found emerging from *C. cinctus*-cut wheat stubble (Morrill et al. 2001b). In addition, larvae of this species have been found inside of *C. cinctus*-infested wheat stubble. It is suspected of feeding on *C. cinctus* larvae, but the exact biology and life history of this predatory beetle are not yet known and require further study.

Braconid Parasitoids of Wheat Stem Sawfly Larvae:

Before the widespread cultivation of wheat on the Great Plains, *C. cinctus* populations in native grasslands existed with a variety of endemic parasitic wasps (Ainslie 1920; Criddle 1922). Once widespread cultivation of wheat began to destroy these native grasslands, *C. cinctus* and their obligate parasitoids migrated into wheat fields (Farstad 1940, Davis 1955, Holmes 1978). While the wheat stem sawfly was able

to quickly adapt and flourish, its braconid parasitoids had greater difficulty in adapting and synchronizing their activities to these new conditions. Over time, some populations of parasitoids were able to adapt to the new location and feeding habits of their host and effectively resumed their utilization of the wheat stem sawfly (Morrill et al. 1998). Though there are nine documented hymenopteran parasitoids of the wheat stem sawfly (Meers 2005), only two have demonstrated substantial ability to affect wheat stem sawfly populations in the field.

Bracon cephi and *B. lissogaster* are two bivoltine braconid parasitoids that are commonly found in wheat fields in Montana today. These two parasitoids were very effective at utilizing wheat stem sawfly populations in native grasslands, but their effectiveness in wheat fields was initially limited (Somsen and Luginbill 1956). There is great variation in the ability of these braconid parasitoid populations to effectively control populations of *C. cinctus* in wheat fields, with reductions in wheat stem sawfly populations ranging from 5 to 85% (Somsen and Luginbill 1956, Morrill et al. 1998, Runyon et al. 2002). Studies to explain this variability have found that the effective parasitism of *C. cinctus* by *B. cephi* is dependent on the synchronization of certain events in the life history of the *C. cinctus* larvae and the *B. cephi* adult (Holmes et al. 1963).

It has been proposed that long-season wheat varieties that stay green long enough to allow the second generation of parasitoids to emerge from the cadavers of *C. cinctus* parasitized in June and continue to parasitize remaining larvae before host plant senescence triggers diapause, or delayed planting that achieves the same effect can result in dramatic decreases in wheat stem sawfly numbers. First-generation parasitoids can be

consumed by other wheat stem sawfly larvae because the first generation females of both *B. cephi* and *B. lissogaster* often parasitize larvae in stems early in the season that have multiple *C. cinctus* larvae in them (Weaver et al. 2005). It has also been reported that first generation parasitism of wheat stem sawfly larvae can have beneficial effects on infested wheat plants, halting the season-long stem mining activities of the larvae before they severely affect the physiology and yield of the plant (Buteler et al. 2008)

Because wheat stem sawfly larvae are cannibalistic, wheat stems with multiple *C. cinctus* eggs often produce only one *C. cinctus* adult. This trait makes it difficult for braconid populations to become established in wheat fields experiencing high numbers of *C. cinctus* larvae per stem because it is possible that the parasitoid larvae are consumed along with their paralyzed host by another *C. cinctus* larva (Weaver et al. 2005). In addition to cannibalism, the amount of stem cutting by *C. cinctus* larvae, soil temperature and moisture, developmental rate of the host plant, and tillage have all been shown to have effects on rates of parasitism (Holmes et al. 1963, Runyon et al. 2002).

Two braconid species, *B. cephi* and *B. lissogaster*, are the only parasitoid species of *C. cinctus* that are found in large numbers in wheat fields on the northern Plains today. Both of these species have similar life histories and occur concurrently in wheat fields across Montana. Once suitable host larvae have been located inside of wheat stems, the female parasitoids pierce the stem with their ovipositors, paralyzing the larvae and laying eggs on the surface of the pest. *Bracon cephi* is known to lay only one egg per larva, while *B. lissogaster* is reported to lay multiple eggs per larva (Nelson and Farstad 1953, Holmes et al. 1963). Parasitized larvae are paralyzed by toxins from the venom gland of

the female, but remain alive to allow the developing parasitoid larvae to feed. After paralysis, herbivory by the wheat stem sawfly ceases, and the girdling of the stem at the base of the plant and the resulting lodging is prevented.

Currently, rates of parasitism are sufficient (approximately 90%) in some areas of Montana that cutting is reduced to a level that is considered economically acceptable (Runyon 2001, Morrill et al. 1998). Though both parasitoid species occur in the same areas in Montana, have very similar life histories, utilize the same host, and are morphologically similar (Runyon et al. 2001), little else is known about the relative population abundances of these two species in wheat fields. Though the existence of two generations of each species per year has been reported (Nelson and Farstad, 1953), there are indications that the second, later generation often has trouble maturing due to drought or excessive heat found in the late summer months (Holmes, 1963).

Dufour's glands in female Hymenoptera house a wide variety of semiochemicals that are responsible for mediating a wide range of behaviors (Ali and Morgan 1990). *Bracon cephi* and *B. lissogaster* are known to respond to a variety of chemical cues to help them locate their hosts and both species respond positively to specific volatile compounds produced by wheat plants that are being injured by *C. cinctus* (Perez 2009). Secretions by Dufour's gland are known to be mediators of oviposition processes and catalysts for courtship behavior (Mudd et al. 1982, Marris et al. 1996, Syvertsen et al. 1995) and it has been suggested that four acetate esters: *n*-tetradecyl acetate, (*Z*)-7-hexadecen-1-yl acetate, (*Z*)-9-hexadecen-1-yl acetate, and (*Z*)-11-hexadecen-1-yl acetate, present in the Dufour's glands of *B. cephi* and *B. lissogaster,* are candidate sex

pheromones which may elicit positive behavioral responses from both of the sexes (Baker et al. 2005). Understanding the roles these compounds may play in mediating behavioral responses of these parasitoids could help elucidate the mechanisms through which these two species maintain reproductive isolation.

Questions of Interest

Because little is known about the population dynamics, chemical ecology, and behavior of these two parasitoid species, the line of reasoning employed in this work relied upon the answers to the following questions of interest to help formulate the subsequent steps of this investigation.

- Do *B. cephi* and *B. lissogaster* maintain reproductive isolation through temporal differences in population abundances?
- Are the four acetate esters identified by Baker et al. (2001) in the Dufour's glands of *B. cephi* and *B. lissogaster* found in significantly different amounts between the two species?
- Do these acetate esters have any physiological or behavioral activity?
- Do males and females of the same species respond differently to males and females of the other species?

Need for Further Research

Ultimately, the goal of the research focusing on characterizing the chemical ecology and behavior of these congeneric parasitoids is the development of further

integrated pest management solutions for *C. cinctus* in the northern Great Plains. Practically speaking, where robust natural populations of these parasitoids are present in Montana, they have been shown to suppress wheat stem sawfly populations (Runyon 2001, Morrill et al. 1998). Because there are slight differences in host selection and parasitism between the two species, and it may be preferential establish one species over the other in a reintroduction plan, practical in-field tests of isolated pheromone components should remain the ultimate goal of research conducted in this area.

Because the low minimum effective population size of the wheat stem sawfly is a combination of haplodiploid reproduction and the relatively large number of eggs carried by each female, mortality has to exceed 90% to begin to have an effect on infestation rates and numbers of individuals in subsequent generations (Holmes 1982). Achieving these mortality rates through any one control tactic may well be impossible; developing a multifaceted approach combining cultural methods with plant resistance, differential cultivar preferences and biological controls is likely to be the most effective strategy for wheat stem sawfly management.

METHODS: FIELD POPULATION ABUNDANCE SURVEY

Field Population Abundance Survey, Summer 2010

Data Collection:

A survey of the populations of *B. cephi* and *B. lissogaster* naturally occurring in wheat fields outside of Conrad and Havre, Montana was conducted from June 8 to August 3 on a weekly basis during the summer of 2010. Sweep nets were used to obtain representative samples of the populations of these species in wheat fields using a grid-based random sampling methodology along the edges of fields where host density was highest (Weaver et al., 2005).

Development of Sampling Regime:

To account for the variability of in-field distribution of adult parasitoids after a pilot study in 2009 that used 20 sweeps per sample and 10 samples per field, the number of sweeps per sample was increased from 20 to 100, and the number of samples per field increased from 10 to 20. Sweeping in a 180 degree arc across the front of the body in one direction was defined as one sweep. Though this was not the optimal number of samples suggested by a Taylor's power law analysis of the 2009 data (which required 1,000 to 5,000 samples to achieve an SE/mean ratio of 0.1 at low parasitoid sampling densities), this sampling regimen represented the upper limit of the field sample handling and identification capacity of the researchers involved.

Ten sweeping sections on the eastern and western edges of the fields were chosen randomly each week for a total of 20 sections swept per field per week. A large “W”

pattern was swept through each 20 x 20 meter section, starting at one outside corner, proceeding to the inner edge, traveling back to the outside edge, then returning to the inside edge and finally finishing at the opposite outside corner.

Site Descriptions:

During the summer of 2010, two fields outside of Havre, MT and two fields outside of Conrad, MT were sampled weekly. One field at each location was part of a trap-crop experiment, while the other two fields were planted in a standard fallow-crop rotation block system. Sampling in the summer of 2009 indicated that 10 samples of 20 sweeps each provided a marginally acceptable estimate of the abundances of *B. cephi* and *B. lissogaster* in wheat fields near Havre and Conrad, Montana.

Conrad, Phillips 2010: The site was located approximately 12.8 km southwest of Conrad, MT on the Pendroy Road (48º 5' 12.2" N, 112º 4' 0.5" W; Teton County). The trap-crop experiment was organized into six strips of 40 x 137 m separated by a 12.8 m herbicide sprayed flyway. A trap-crop of Choteau variety spring wheat formed a 40 m perimeter around higher yielding Reeder and Conan hollow-stem varieties of spring wheat. Three contiguous 20 x 20 m sweep sections were flagged along the eastern and western edges of each trap-crop strip repetition for a total of 36 sweep sections (18 East, 18 West). Shortly before the harvest date, the total numbers of females of both species collected from each sweep section were tallied and the four sweep sections on each side of the field with the highest and lowest number of female parasitoids were sampled by pulling five random 0.33 row-meter wheat samples from each section. These samples

were then split and the number of parasitoid cocoons tallied for each of the sampled sections.

Conrad, Spears 2010: The site was located approximately 8 kilometers south of Conrad, MT on Pendroy Road (48º 8' 19.7" N, 111º 58' 49.4" W; Pondera County). The field consisted of two monoculture blocks of Choteau spring wheat. Due to the borders being bound by grassland, the eastern and western edges of the two blocks, separated by 200 m of fallow from the previous year's wheat crop, were flagged with 20 contiguous 20 x 20 m sweeping sections for a total of 40 sweep sections.

Havre, Peterson 2010: The site was located approximately 45 km north of Havre, MT off of Highway 232 (48º 50' 9.8" N, 110º 5' 41.2" W; Hill County). The trap-crop was organized into six strips of 40 x 137 m separated by a 12.8 m herbicide sprayed flyway with a trap-crop of Choteau variety spring wheat forming a 50 m perimeter around higher yielding Reeder and Conan hollow-stem varieties of spring wheat. Three contiguous 20 x 20 m sweep sections were flagged along the eastern and western edges of each trap-crop strip repetition for a total of 36 sweep sections (18 East, 18 West). Shortly before the harvest date, the total numbers of female parasitoids of both species collected from each sweep section were tallied and the four sweep sections on each side of the field with the highest and lowest number of female parasitoids were sampled by pulling five random 0.33 row-meter wheat samples from each section. These samples were then split and the number of parasitoid cocoons tallied for each of the sampled sections.

Havre, Hockett 2010: The site was located approximately 16 km south of the Montana State University Northern Agricultural Research Center off of Highway 87 (48º 28' 50.7" N, 109º 53' 51.2" W; Hill County). The field was a homogeneous block of Choteau spring wheat. Twenty-eight contiguous 20 x 20 m sweeping sections were flagged along the eastern and western edges of the field for a total of 56 sweep sections.

Field Population Abundance Survey, Summer 2011

Data Collection:

A survey of the populations of *B. cephi* and *B. lissogaster* naturally occurring in wheat fields outside of Conrad and Amsterdam, Montana was conducted from June 17 to August 19 on a weekly basis during the summer of 2011. Sweep nets were again used to obtain representative samples of the populations of these species in wheat fields using a grid-based random sampling methodology along the edges of fields where host density was highest.

Site Descriptions:

During the summer of 2011, two fields outside of Conrad, MT and one field outside of Amsterdam, MT were sampled weekly. All three fields were planted in a standard fallow-crop block rotation system. Ten sweeping sections on the eastern and western edges of the fields were randomly sampled each week for a total of 20 sections sampled per field per week. A large "W" pattern was swept through each 20 x 20 m section, starting at one outside corner, proceeding to the inner edge, traveling back to the

outside edge, then returning to the inside edge to finish at the opposite outside corner. On average, it took 100 sweeps to complete each sweeping section.

Conrad, Phillips 2011: The site was located approximately 12.8 km southwest of Conrad, MT along Pendroy Road (48º 5' 12.2" N, 112º 4' 9.6" W; Teton County). The western edge of the field was adjacent to a narrow 3 m native grass strip underneath a power transmission line, with fallow on the other side of the strip. The eastern edge of the field bordered fallow that had been sampled using the same methods the previous year (Phillips SARE trap-crop experiment). Eighteen contiguous 20 x 20 m sweeping sections were flagged along the eastern and western borders of the field, for a total of thirty-six sweeping sections (18 East, 18 West). Sweep sampling was executed using the same methodology used in 2010. Shortly before the harvest date, the total female parasitoids of both species collected from each sweep section were tallied and the four sweep sections on each side of the field with the highest and lowest number of female parasitoids were sampled by pulling five random 0.33 row-meter wheat samples from each section. These samples were then split and the number of parasitoid cocoons tallied for each of the sampled sections.

Conrad, Nelson 2011: The site was located approximately 1.6 km south of the junction of state highway 219 and 8th Lane NW (48º 3' 30.3" N, 112º 10' 31.3" W; Teton County). The eastern and western edges of the field were bordered by fallow from the previous year, with active oil wells and a collecting station within 0.4 kilometers on the same property. The field was planted as a monolithic block of Corbin spring wheat.

Eighteen contiguous 20 x 20 m sweeping sections were flagged along the eastern and western borders of the field, for a total of thirty-six sweeping sections (18 East, 18 West). Sweep sampling was executed using the same methodology used in 2010. Shortly before the harvest date, the total numbers of females of both species collected from each sweep section were tallied and the four sweep sections on each side of the field with the highest and lowest number of female parasitoids were sampled by pulling five random 1 row-foot wheat samples from each section. These samples were then split and the number of parasitoid cocoons tallied for each of the sampled sections.

Amsterdam, Bates 2011: The site was located approximately 5.6 km west of Amsterdam, MT along Amsterdam Road (45º 45' 32.3" N, 111º 22' 20.5" W; Gallatin County). The western edge of the field was bordered by a small horse pasture with native grasses, while the eastern edge was bordered by a potato field that had been used to grow wheat the year before. The southern edge was bordered by a dirt road and another crop of spring wheat. The northern edge was bordered by fallow from the previous year's crop. The field was planted in a monolithic block of an herbicide resistant Clearfield (BASF) line of spring wheat. Eighteen contiguous 20 x 20 m sweeping sections were flagged along the northern and eastern borders of the field, for a total of thirty-six sweeping sections (18 East, 18 West). Sweep sampling was executed using the same methodology used in 2010. Shortly before the harvest date, the total female parasitoids of both species collected from each sweep section were tallied and the four sweep sections on each side of the field with the highest and lowest number of female parasitoids were sampled by

pulling five random 1 row-foot wheat samples from each section. These samples were then split and the number of parasitoid cocoons tallied for each of the sampled sections.

Field Population Abundance Survey, 2010-2011 Data Analysis

The relative densities of *B. cephi* and *B. lissogaster* for each field site for both years were visually explored by plotting abundance as a function of time using side-by-side boxplots for each species. For fields where harvest wheat samples were taken from the four highest and lowest female parasitoid-yielding sweep sections on each side of the field, an attempt was made to correlate the number of cocoons found in the harvest samples with the number of female parasitoids collected during the course of the summer in those particular sweep sections. The percent parasitism of larvae and the number of females per sweep was determined, and the Mantel test (Mantel, 1967) was used to test the correlation between the number of cocoons and number of female parasitoids collected at each of the sampled sweep sections in each field.

METHODS: DUFOUR'S GLAND CHEMICAL ANALYSIS

Data Collection

The chemical composition of the contents of the Dufour's gland for each species of braconid parasitoid was determined using gas chromatography and mass spectrometry. Multiple individual females collected from wheat fields outside of Havre and Conrad, MT in the summer of 2011 supplemented by those emerging from cocoons in wheat residue collected in the spring and fall of 2010 were dissected, removing the Dufour's glands outlined in Baker et al. (2005). Each individual gland was punctured with a drawn glass capillary needle similar to the method described by Morgan (1990) and immersed in 160 µl of hexane contained in a glass auto-injector vial. A 50-µl aliquot of a 7.3 ng/µl concentration of nonyl acetate was then added to the 160 µl solution as an internal standard.

The resulting supernatant of the glandular solution was then transferred to a clean vial using a "u" shaped glass capillary tube. Positive pressure exerted by the evaporation of hexane inside the sealed auto-injector glass vial pumped the supernatant into an empty vial, separating the fluid from the glandular tissue in the first vial and minimizing risk of damage to the gas chromatography column by injection of tissue microparticles. The solution was then evaporated under a high purity nitrogen stream to 25% of original volume to concentrate the contents of the gland. Four of these resulting single-gland solutions were then combined into one solution in a glass auto-injector vial.

The solution was analyzed using an Agilent 6890 series Gas Chromatograph (Agilent Technologies) equipped with a high resolution gas chromatography column (J and W Scientific HP-5MS, 30m x 0.25mm ID, 0.25µm film, Fulsom, California). Column head pressure was maintained at approximately 71.71 kPa using ultra-pure helium as the carrier gas, with a starting temperature of 50 °C, a ramp rate of 5 °C degrees per minute, and a finishing temperature of 300 °C. The final temperature was held for 12 minutes, followed by a cool down and hold period of 15 minutes at 50 °C. The GC/MS interface was maintained at 200 °C. The mass selective detector used was an Agilent 5973 (Agilent Technologies).

Data Analysis

The chromatograms obtained from the Dufour's gland elutions were analyzed using the data analysis program of Agilent Technologies ChemStation software package. Acetate esters were identified by retention time and comparison to mass spectra of synthetic acetate esters. Using integration parameters determined by the amount of baseline variation in the traces from the Dufour's gland samples, the areas of the peaks for all the acetate esters were compared to the areas of the nonyl acetate internal standard, as well as to the areas of synthetic acetate esters of known concentration.

Synthetic acetate esters at varying concentrations were run through the same temperature and method GC/MS program, and the relationship between peak area and concentration was regressed using TableCurve 2D (version 5.01, SyStat Software Inc., 2002). These regressions established the relationship between peak area and

concentration for each acetate ester. The peak area values of acetate ester obtained from running the Dufour's gland solutions of females of both species through the GC/MS program were then integrated using the appropriate regression and the resulting concentration determined.

Concentrations of the gland acetate esters for each species were compared and the ratios of the acetate esters present in the Dufour's glands of the females of each species were calculated.

METHODS: ELECTROPHYSIOLOGY OF CANDIDATE SEX PHEREMONES

Data Collection

The electroantennogram (EAG) is a technique used to explore the voltage depolarization of insect antennae in response to chemical stimuli to determine electrophysiological sensitivity to specific compounds. Schneider (1957) used this method on the antennae of male silk moths (*Bombyx mori* L.), which consisted of recording the sums of the electrical potentials produced by the receptor neurons in the antenna of the moth in response to chemical stimuli (Eltz and Lunau 2005). This method provides a convenient way for a wide variety of chemicals and compounds of varying concentrations to be tested for sensitivity in insect antennae (Thiery and Marion-Poll 1998).

Antennal Preparation:

The majority of the preparations of the parasitoid antennal complexes for electrophysiological work were identical to those used by Perez (2009). The heads from *B. lissogaster* and *B. cephi* specimens were removed, and one of the antennae was excised from the head. Using fine micro-dissection scissors, 1/3 of the side of the head with the removed antenna was sectioned to expose the antennal nerves and improve electrical conductance. The parasitoid head was placed on a gold electrode (conducting) and the tip of the antenna placed on another gold electrode (recording). Electrical

conductance between the electrode surface and the antennal nerve was facilitated with an electrolytic gel (Spectra 360, Parker Laboratories, Inc., Fairfield, New Jersey).

A less invasive technique using a drawn glass capillary needle filled with a dilute 0.9% NaCl solution inserted into the base of the parasitoid head was also used when testing response to a series of higher concentration acetate esters and volatile plant compounds. The drawn glass capillary needle was fitted over a wire electrode, with electrical conductance between the antennal nerves and the conducting electrode provided by the 0.9% saline solution, similar to the methods used by Chen and Fadamiro (2007). One antenna was removed, and the remaining antenna was connected to the recording electrode which consisted of an identical drawn glass capillary tube filled with saline solution.

Recording Equipment:

The two electrodes were attached to a micro-manipulator, type INR-5 (Narishige MN-151). The micromanipulator was connected to a serial-data acquisition interface amplifier, type IDAC-232 (Syntech Hilversum, The Netherlands), which stabilized the input signal from the parasitoid antenna and amplified it. The amplifier transferred the signal to a computer interface running EAG Pro version 1.1 software (Syntech, © 2003-2007, Kirchzarten, Germany). This apparatus is commonly used as the standard for EAG research (Birkett et al. 2004).

Applied Stimuli:

Four acetate esters (*n*-tetradecyl acetate, (*Z*)-7-hexadecen-1-yl acetate, (*Z*)-9-hexadecen-1-yl acetate, and (*Z*)-11-hexadecen-1-yl acetate) identified in the Dufour's gland solutions of *B. cephi* and *B. lissogaster* were used to stimulate the antennae of male *B. cephi* and *B. lissogaster*. Serial dilutions in hexane of each of the acetate esters were used to deliver 1, 10, 100, 1,000, and 10,000 ng doses in a 10 µl aliquot to test the electrophysiological response of male parasitoid antennae to a wide range of concentrations. The concentrations of acetate esters tested were diluted from stock solutions obtained from ISCA Technologies (Riverside, CA) and Bedoukian Research (Danbury, CT).

A 10 µl aliquot of each solution was applied to a piece of filter paper (Whatman® no. 2) located inside a glass Pasteur pipette. After allowing one minute for solvent evaporation, the pipette tip was placed into a small 3-mm hole in a glass tube carrying a continuous stream of humidified air towards the antennal preparation located in the opening of the tube. The Pasteur pipette was connected to an air stimulus controller which delivered a 0.2 second puff of air through the impregnated filter paper and into the humidified air stream. The resulting change in voltage between the two electrodes was then recorded. Each odor stimulus that was part of a serial dilution in hexane was followed by a puff of a 10 µl aliquot of hexane as a control. In later experiments, an air control puff was added as a control for tests of pure acetate esters and plant compounds that were not diluted in hexane. Finally, pieces of filter paper impregnated with a 1 µl aliquot of the stock compounds were placed in close proximity (approximately 1 cm) to

the antenna and then removed over the course of two seconds to examine the response to acetate ester diffused from localized sources.

Data Analysis

After numerous replications, the mean maximum peak voltage depolarization for each of the acetate esters were calculated for each concentration tested. The mean depolarizations for the pure acetate ester and plant compound trials were also calculated.

METHODS: BEHAVIORAL ASSAYS OF LIVE INSECTS

Data Collection

Hand-blown glass Y-tubes are often used in behavioral choice tests for insects. By placing an odor source in one branch of the "Y" and a control in the other, air moving down both branches allows an insect traveling up the long arm of the y-tube can make a choice when it reaches the fork to either continue towards the odor source or to continue toward the control. Lighting often is effective in triggering movement in many phototaxic insects, but requires careful balancing and alternation of the sides of the "Y" that control and odor sources are presented to minimize any potentially confounding effects.

Insect Preparation:

Live insect stimuli with live insect responders were used in behavioral choice tests using Y-tubes. These were conducted between January 27, 2012 and June 4, 2012, with both males and females of both species being used as odor sources and responders. For the odor sources, 10 newly emerged, unmated individuals of one species and sex were isolated from each other for 24 hours before exposure. The responders, also newly emerged and unmated individuals, were individually isolated for 24 hours in glass vials before exposure to the odor source. The odor-source individuals were then placed together in a quartz vial with fritted glass plugs on either end to allow air to pass through from one side to the other, but restrict insect movement out of the vial. This odor-source vial was then instead into the airstream for one branch of the Y-tube. An identical quartz

vial without any insects was used as the control and placed in line with the airstream for the opposite branch of the Y-tube.

Y-tube Conditions:

The humidified air stream supplying both branches of the Y-tube flowed at a rate of 0.5 L/min. The odor source and control quartz vials alternated locations after every Y-tube replication to minimize the potentially confounding effects of minute differences in light intensity on either side of the Y-tube centerline resulting in slight phototaxic preferences for one side of the Y-tube or another. Ambient air temperature was maintained at 29.4^{o} C and isolated responder insects before use were exposed to a bright halogen light for 10 seconds to increase their activity level before being introduced into the Y-tube. Fresh Y-tubes were used after each individual insect trial to prevent responders from following potential chemical trails laid down by previous responders.

Mating Trials:

Live insect mating trials were also conducted between February 6, 2012 and April 3, 2012, with newly emerged males and females of both species being paired together in glass shell vials with a moistened cotton plug. Before exposure, insects were kept isolated for 24 h in glass shell vials with a moistened cotton plug at 4.4° C. Insect pairs were placed in close proximity (8 cm) to a bright fluorescent light to increase activity levels and observed for 1 hour. Mating attempts, successful copulation, and mating-type behavior were all recorded and tallied for each male-female pairing.

Data Analysis

A two-way chi-square test was used to test for significance when comparing odor source vs. control choices made by responder insects for both the acetate ester Y-tube trials and the live insect Y-tube trials. Though the experiments were conducted in an environmentally controlled laboratory setting, rapid changes in barometric pressure had a negative effect on the activity levels and response rates of the parasitoids in behavioral assays. Therefore, assays conducted from January 27, 2012 to April 30, 2012 where the barometric pressure precipitously dropped (below 100.9 kPa) in correlation with an advancing storm front or depression were removed from the data set.

For the live insect mating trials, mating attempts (physical contact, mounting by male), copulation and mating-type behavior (wing fanning, greater activity, and rapid antennal tapping in proximity of the female) formed the response categories. The number of successful copulation, mating attempts and mating-type behavior for each mating pair were summed for each species pairing.

RESULTS: FIELD POPULATION ABUNDANCE SURVEY

Sequential Field Abundance Sampling of Parasitoids

Temporal sampling data from the summers of 2010 and 2011 indicate that temporal overlap does occur between the populations of *B. cephi* and *B. lissogaster* in wheat fields sampled outside of Havre, Conrad and Amsterdam, MT. (Figures 1, 2). Additionally, data from certain fields suggest the possibility of the occurrence of two temporally separated generations of *B. lissogaster* and *B. cephi* during the course of the summers, with the potential second generation being smaller (Figures 1, 2).

Percent Parasitism as a Function of Number of Female Parasitoids

Of the four sites sweep-sampled in 2010, two (Havre-Peterson and Conrad-Phillips) were selected for percent parasitism analysis of harvest samples (Figures 3, 4). Out of the three sites sweep-sampled in 2011, all were selected for analysis of percent parasitism (Figures 5-7). There were no significant correlations between the number of females collected by sweep sampling and parasitism rates for any of the years or fields (Figure 4).

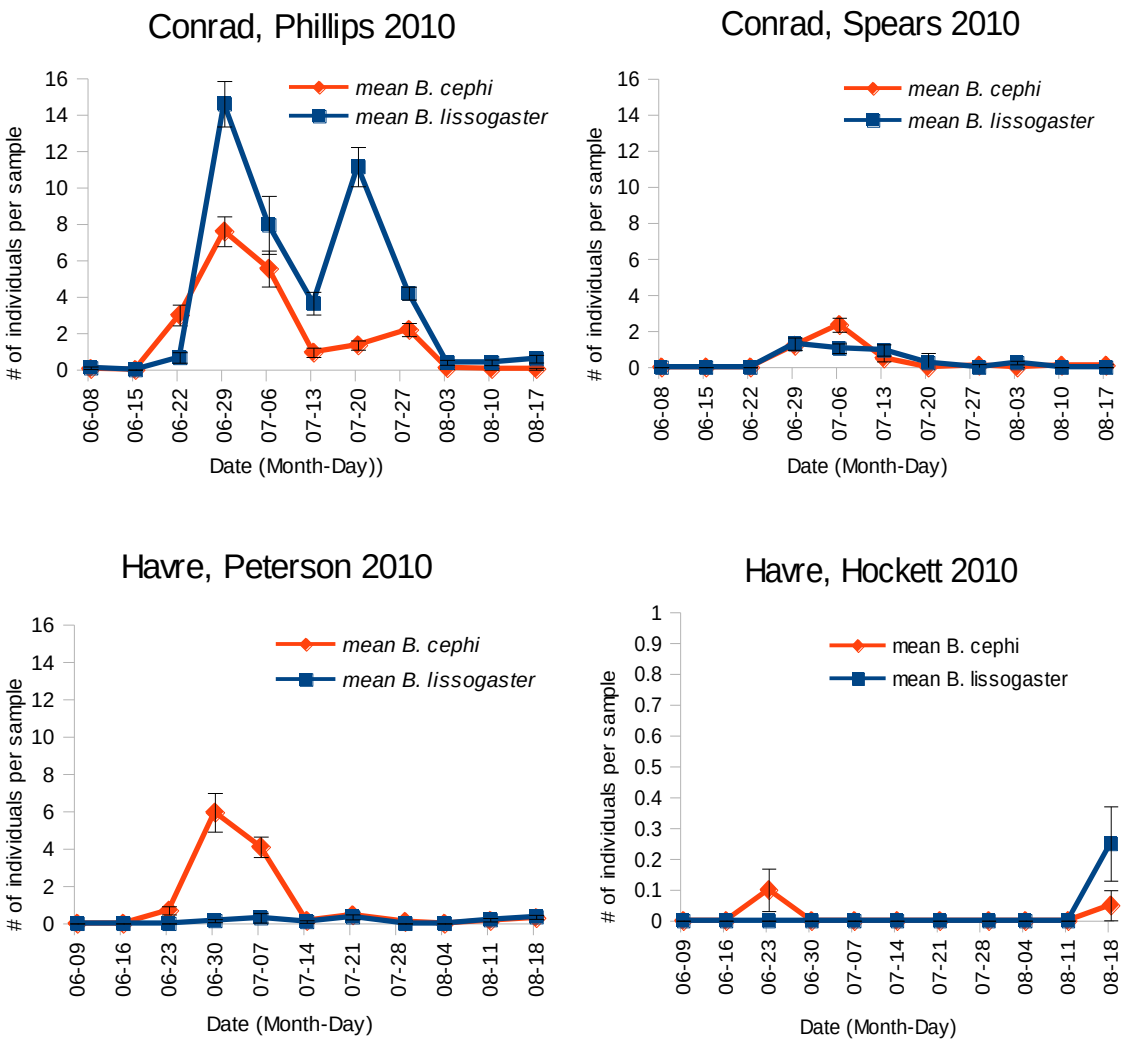


Figure 1. Summer 2010 number of individuals (both sexes) per sample (n = 20) of *B. cephi* and *B. lissogaster* as a function of time. Sampling was conducted weekly, starting June 8 and ending August 3 for a total of 9 weeks (except Conrad, Phillips, whose sampling continued for a 10th week). Data points missing for certain weeks represents days where sweep-net sampling was not possible due to rain.

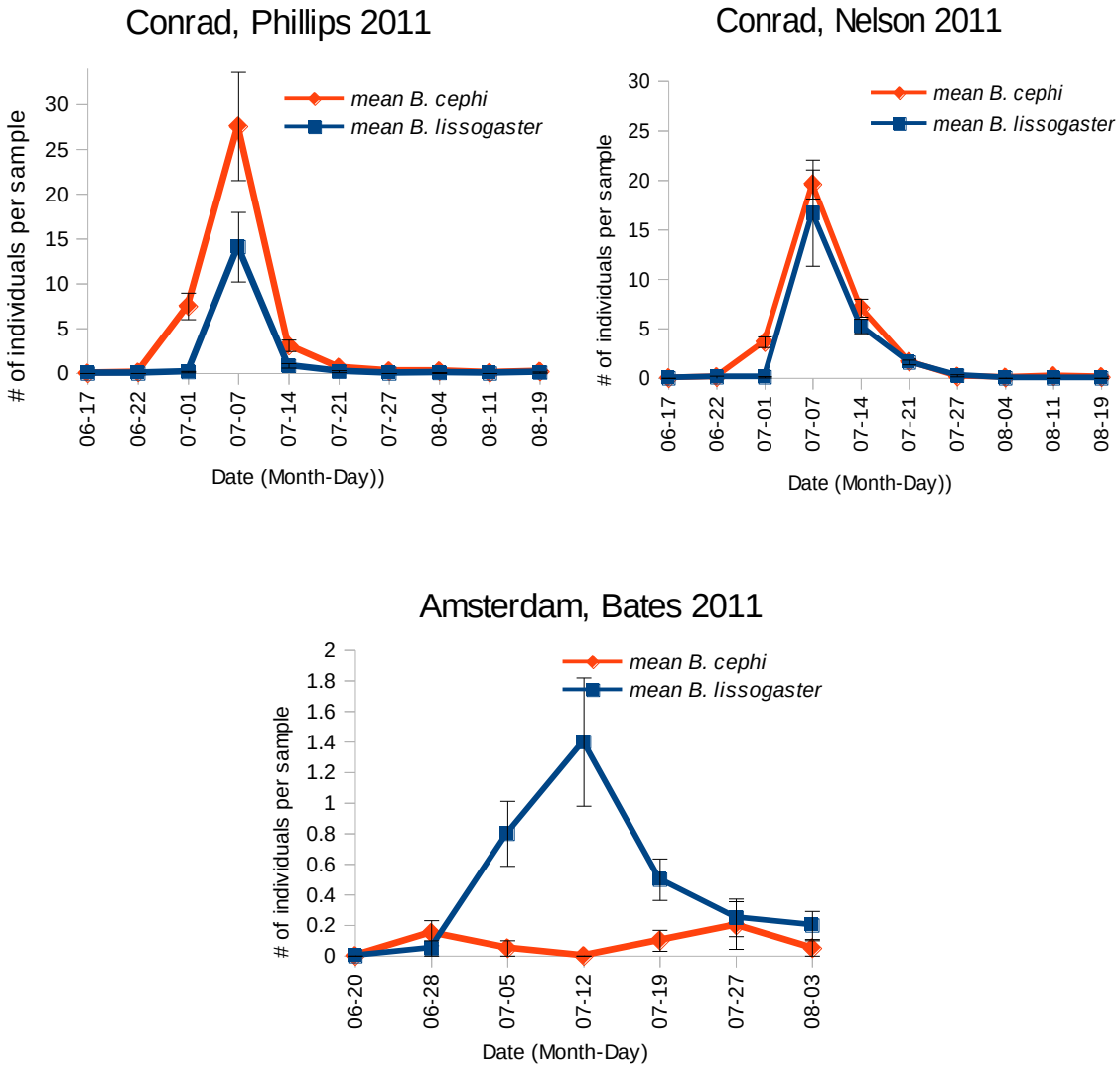
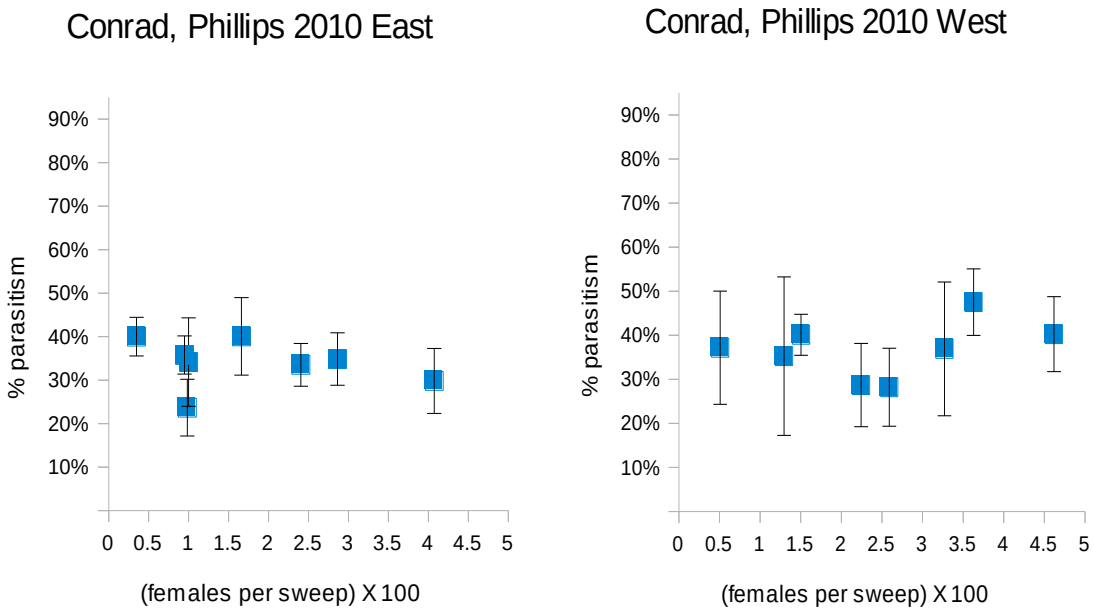
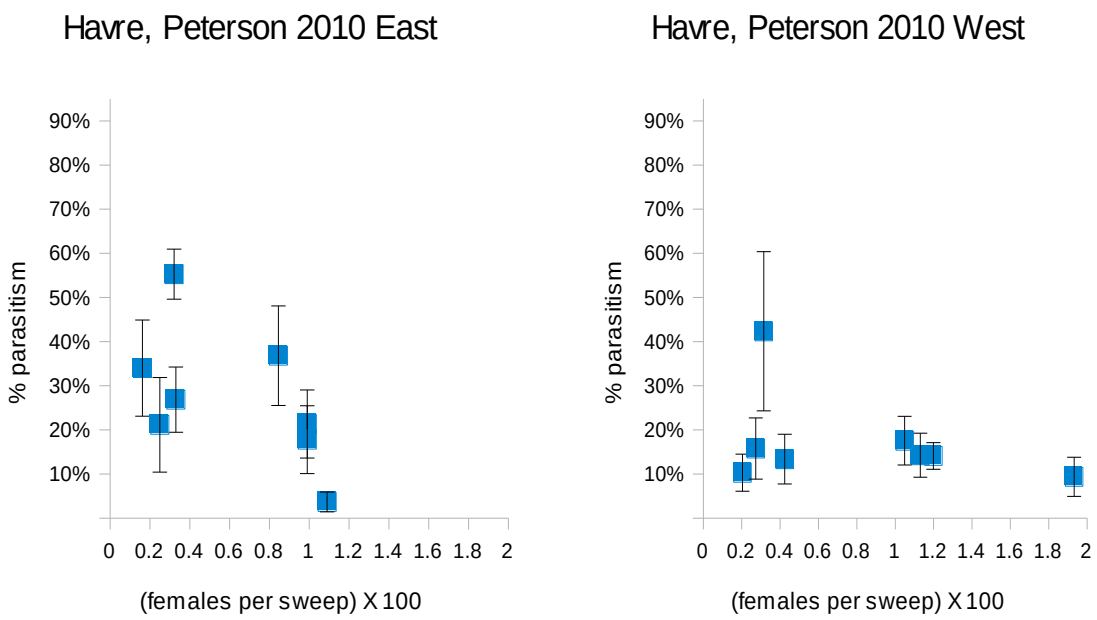


Figure 2. Summer 2011 number of individuals (both sexes) per sample (n = 20) of *B. cephi* and *B. lissogaster* as a function of time. Sampling was conducted weekly, starting June 17 and ending August 19 for a total of 10 weeks (except Amsterdam, Bates, where sampling continued for seven weeks).



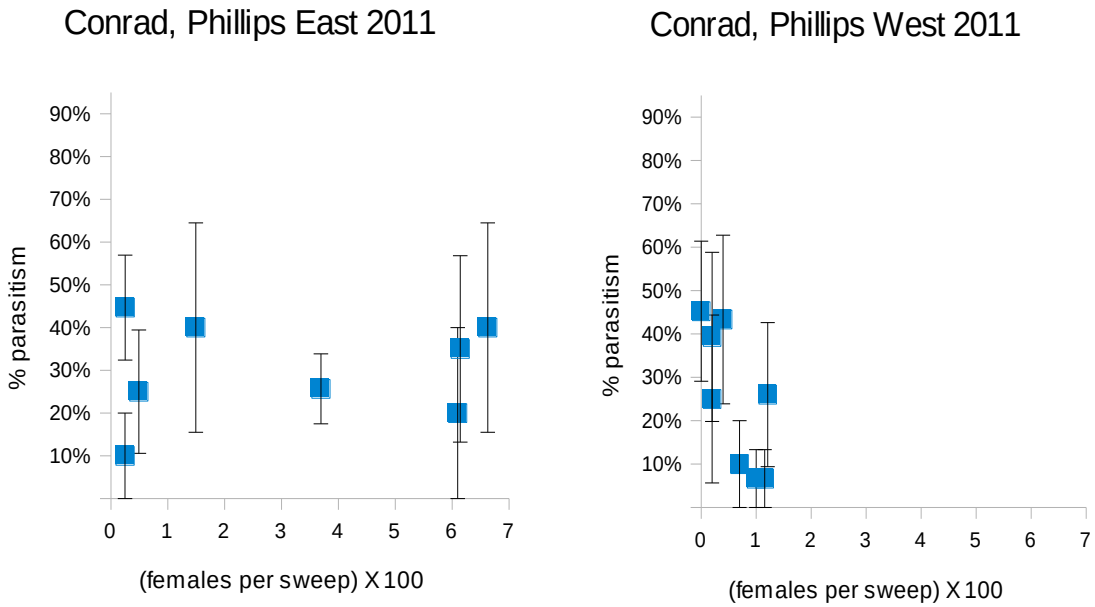
Mantel test for correlation between % parasitism and (females per sweep) X 100 Based on 9999 replicates Simulated p-value: 0.53	Mantel test for correlation between % parasitism and (females per sweeps) X 100 Based on 9999 replicates Simulated p-value: 0.73

Figure 3. Conrad, Phillips 2010. Percent parasitism as a function of average number of females per sweep (X 100) for each of the 16 sampled sections (8 per side of field). For each section sampled for % parasitism analysis, n=5.



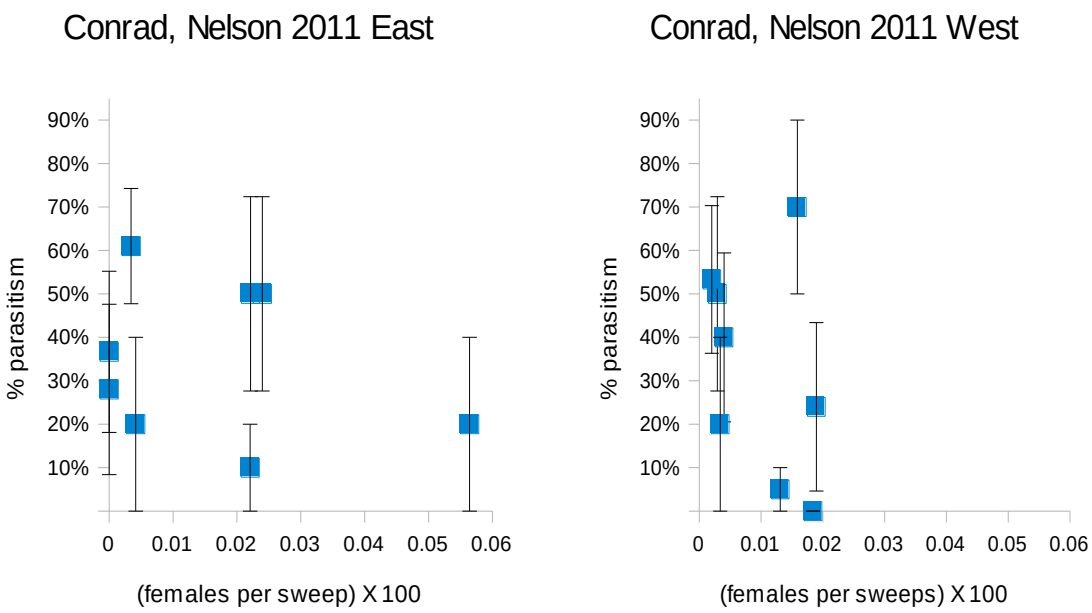
Mantel test for correlation between % parasitism and (females per sweep) X 100 Based on 9999 replicates Simulated p-value: 0.41	Mantel test for correlation between % parasitism and (females per sweep) X 100 Based on 9999 replicates Simulated p-value: 0.40

Figure 4. Havre, Peterson 2010. Percent parasitism as a function of average number of females per sweep (X 100) for each of the 16 sampled sections (8 per side of field). For each section sampled for % parasitism analysis, n=5.



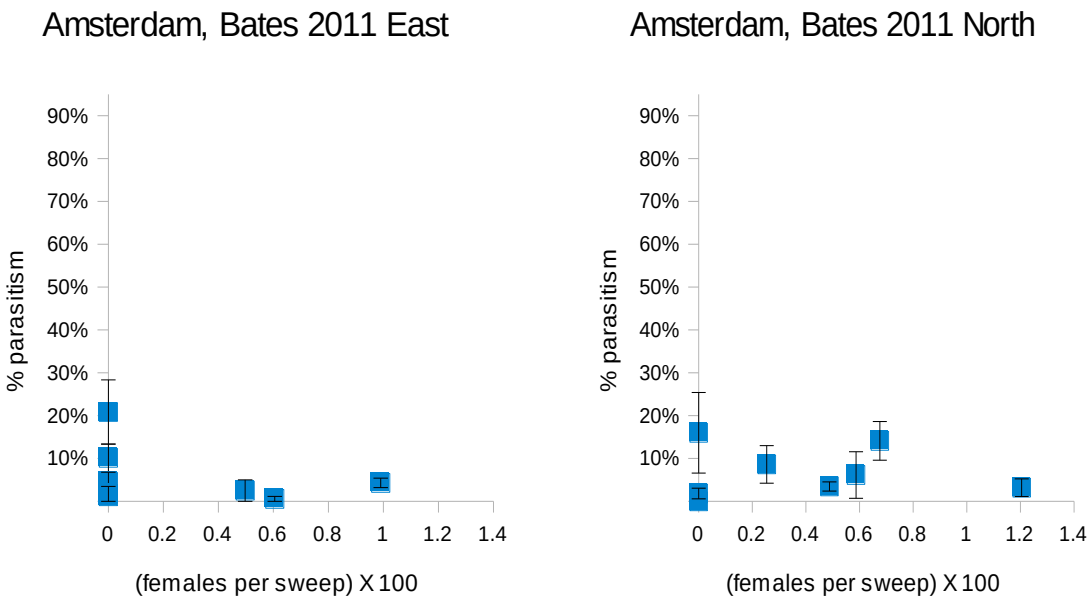
Mantel test for correlation between % parasitism and (females/sweeps) X 100 Based on 9999 replicates Simulated p-value: 0.88	Mantel test for correlation between % parasitism and (females/sweeps) X 100 Based on 9999 replicates Simulated p-value: 0.07

Figure 5. Conrad, Phillips 2011. Percent parasitism as a function of average number of females per sweep (X 100) for each of the 16 sampled sections (8 per side of field). For each section sampled for % parasitism analysis, n=5.



Mantel test for correlation between % parasitism and (females per sweep) X 100 Based on 9999 replicates Simulated p-value: 0.82	Mantel test for correlation between % parasitism and (females per sweep) X 100 Based on 9999 replicates Simulated p-value: 0.28

Figure 6. Conrad, Nelson 2011. Percent parasitism as a function of average number of females per sweep (X 100) for each of the 16 sampled sections (8 per side of field). For each section sampled for % parasitism analysis, n=5.



Mantel test for correlation between % parasitism and (females per sweep) X 100 Based on 9999 replicates Simulated p-value: 0.81	Mantel test for correlation between % parasitism and (females per sweep) X 100 Based on 9999 replicates Simulated p-value: 0.77

Figure 7. Amsterdam, Bates 2011. Percent parasitism as a function of average number of females per sweep (X 100) for each of the 16 sampled sections (8 per side of field). For each section sampled for % parasitism analysis, n=5.

RESULTS: DUFOUR'S GLAND CHEMICAL ANALYSIS

Several Dufour's gland solutions were collected and processed using the GC/MS program described above. Because each solution contained pooled samples of four glands from four different female parasitoids, the amounts and ratios of the acetate esters in the glands were averaged across the solution. With n = 4 for each solution, the solution that yielded the cleanest chromatography for each species was selected for analysis and quantification. Acetate esters identified in these Dufour's gland solutions of female *B. cephi* and *B. lissogaster* using gas chromatography and mass spectrometry corroborated the findings of Baker et al. (2005). The presence of four acetate esters (*n*-tetradecyl acetate, (*Z*)-7-hexadecen-1-yl acetate, (*Z*)-9-hexadecen-1-yl acetate, and (*Z*)-11-hexadecen-1-yl acetate) was confirmed by retention time and comparison with mass spectra of known standards. Use of peak area response curves for each of the acetate esters (Figure 8) allowed the integration of the Dufour's gland data within an existing regression to determine absolute amounts of each acetate ester found in the solutions (Figure 9). The amounts of these acetate esters found in the Dufour's gland solutions varied between the two species, with (*Z*) -11-hexadecen-1-yl acetate only being found in the Dufour's gland of *B. lissogaster* (Figure 9).

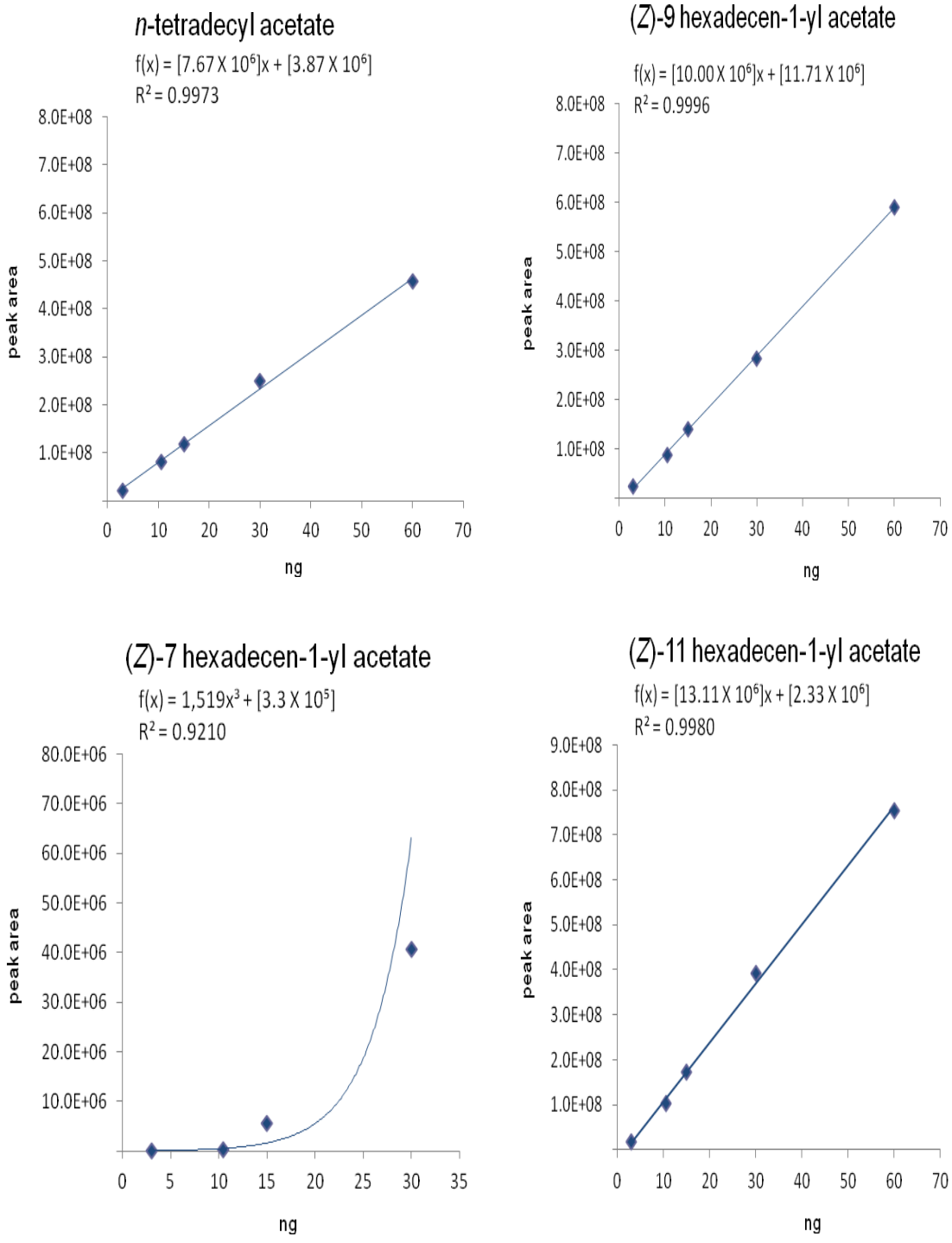
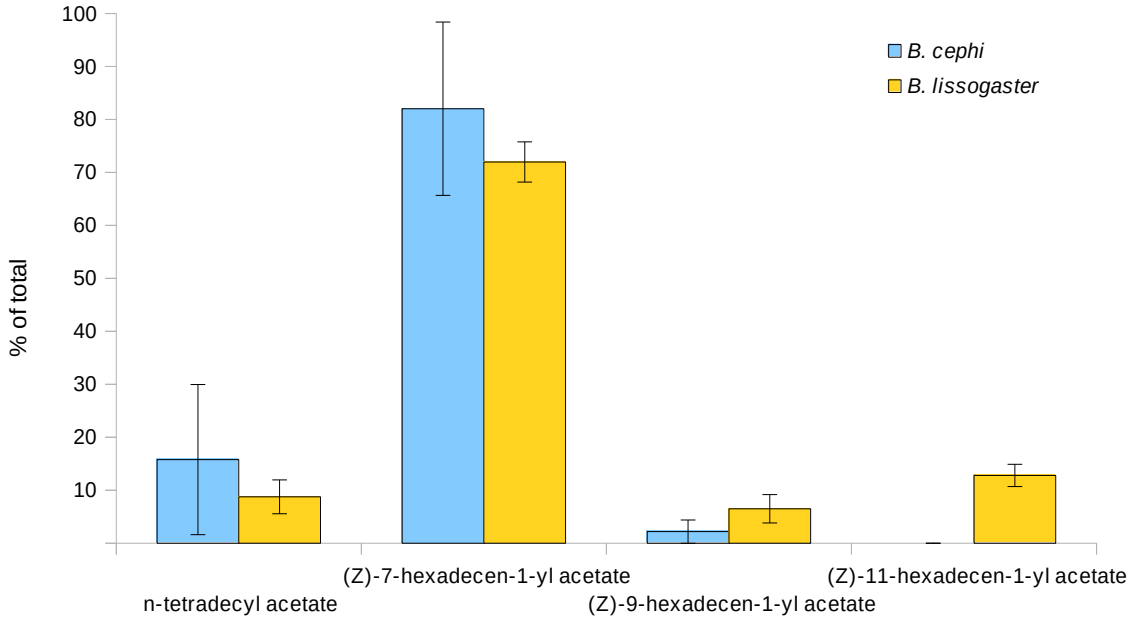


Figure 8. Acetate ester response curves. Mass spectrometer peak area as a function of number of ng in an injected 3 µl solution.



	B. cephi		***B. lissogaster***	
compound	**ng/µl**	**+/-**	**ng/µl**	**+/-**
n-tetradecyl acetate	0.68	0.62	0.56	0.25
(Z)-7-hexadecen-1-yl acetate	3.06	0.21	3.76	0.49
(Z)-9-hexadecen-1-yl acetate	0.1	0.1	0.42	0.2
(Z)-11-hexadecen-1-yl acetate	-	-	0.63	0.01

Figure 9. Percent ratio of acetate esters to each other in Dufour's gland solutions of *Bracon cephi* (n = 4) and *B. lissogaster* (n = 3).

RESULTS: ELECTROPHYSIOLOGY OF CANDIDATE SEX PHEREMONES

Electroantennography of serial dilutions of synthetic acetate esters (*n*-tetradecyl acetate, (*Z*)-7-hexadecen-1-yl acetate, (*Z*)-9-hexadecen-1-yl acetate, and (*Z*)-11-hexadecen-1-yl acetate) indicated that there were no significant dose-dependent increases in the mean electrophysiological activity in the antennae of male *B. cephi* and *B. lissogaster* (Figure 10). In addition, there seemed to be no significant difference in the response of male *B. cephi* and *B. lissogaster* antennae to different acetate esters (Figure 10) when compared with depolarizations associated with plant volatile compounds. The compounds selected for comparison are known to be released by wheat plants under injured by *C. cinctus* larvae (Perez 2009), the depolarizations associated with acetate esters were clearly smaller (Figure 11).

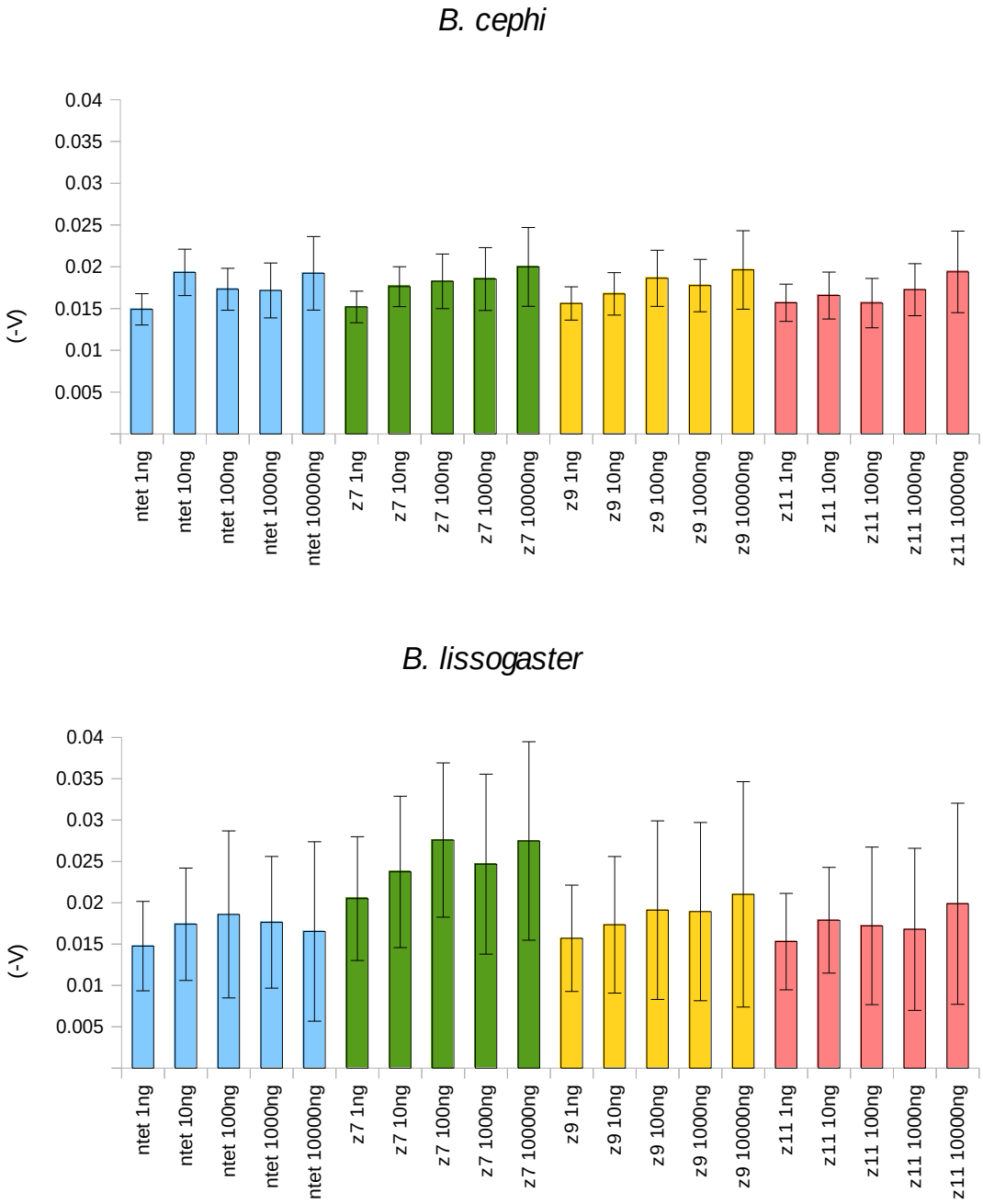


Figure 10. Voltage depolarization of male *B. cephi* (n = 14) and *B. lissogaster* (n = 7) antennae as a function of air puff stimulation with various acetate esters at different dose amounts. Acetate esters are abbreviated (ntet = *n*-tetradecyl acetate, z7 = (*Z*)-7-hexadecen-1-yl acetate, z9 = (*Z*)-9-hexadecen-1-yl acetate, and z11 = (*Z*)-11-hexadecen-1-yl acetate).

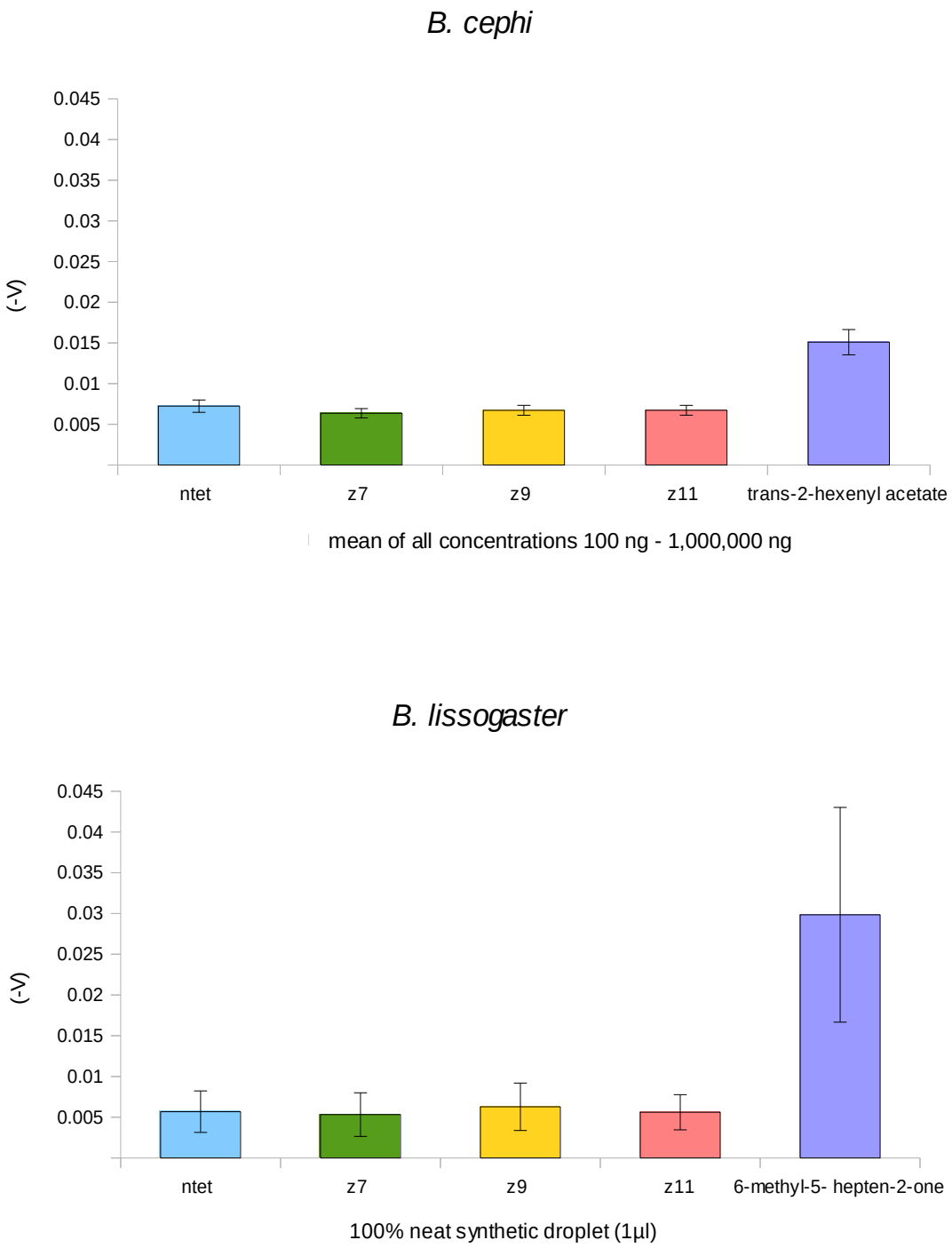


Figure 11. Voltage depolarization of male *B. cephi* (n = 14) and *B. lissogaster* (n = 4) antennae as a function of air puff stimulation with various acetate esters and volatile plant injury compounds.

RESULTS: BEHAVIORAL ASSAYS OF LIVE INSECTS

All possible permutations of odor sources and responders using both sexes of the two species were used in the Y-tube assays. There were significant differences ($p < 0.05$) in the choices made for: female *B. cephi* sources by female *B. lissogaster* (repelled), male *B. lissogaster* sources by male *B. lissogaster* (repelled), and male *B. lissogaster* sources by female *B. cephi* (attracted) (Table 1). Choices made for: female *B. cephi* sources by male *B. cephi* (attracted), male *B. cephi s*ources by female *B. cephi* (attracted), female *B. lissogaster* sources by male *B. lissogaster* (attracted), and male *B. lissogaster* sources by female *B. lissogaster* (repelled) were significant at p = 0.11-0.13; all other pairings were not significant (p = 0.41-0.65) (Table 1).

The isolated insect mating trials indicate that same-species pairings result in mating attempts, mating-type behavior, and successful copulation 22/35 times for male/female *B. cephi* pairings, and 16/38 times for male/female *B. lissogaster* pairings (Table 2). Cross-species pairings resulted in no successful copulation. However, there was one attempted mating out of 13 trials for the female *B. lissogaster*/male *B. cephi* pairing (Table 2). One might expect that copulatory apparatus would be limiting as illustrated by Brajkovic et al. (2010) in Figure 12, but in this data (Table 2) it is obvious that there was only a single unsuccessful mating attempt in many trials across species and that there was very limited activity when the individuals were paired this way. The data for within species pairings are dramatically different.

Table 1. Y-tube choice test matrix for different combinations of *B. cephi* and *B. lissogaster* male and females as odor sources (10 individuals) and responders (1 individual).

Source		Responder		Choice			Chi-squared	p	Behavior
sex	species	sex	species	Air	Source	No Response			
F	*cephi*	F	*cephi*	12	16	2	0.67	0.41	
F	***cephi***	**M**	***cephi***	**14**	**23**	**3**	**2.25**	**0.13**	**Attracted to F *cephi***
F	***cephi***	**F**	***lissogaster***	**18**	**7**	**0**	**4.84**	**0.03**	**Repelled by F *cephi***
F	*cephi*	M	*lissogaster*	11	12	2	0.2	0.65	
M	*cephi*	M	*cephi*	11	14	0	0.36	0.55	
M	***cephi***	**F**	***cephi***	**8**	**16**	**1**	**2.6**	**0.11**	**Attracted to M *cephi***
M	*cephi*	F	*lissogaster*	14	11	0	0.36	0.55	
M	*cephi*	M	*lissogaster*	13	10	2	0.52	0.47	
F	*lissogaster*	F	*lissogaster*	17	13	0	0.53	0.47	
F	***lissogaster***	**M**	***lissogaster***	**8**	**16**	**1**	**2.6**	**0.11**	**Attracted to F *lissogaster***
F	*lissogaster*	M	*cephi*	10	14	1	0.68	0.41	
F	*lissogaster*	F	*cephi*	11	113	1	0.2	0.65	
M	***lissogaster***	**M**	***lissogaster***	**15**	**9**	**1**	**1.48**	**0.04**	**Repelled by M *lissogaster***
M	***lissogaster***	**F**	***lissogaster***	**22**	**13**	**0**	**2.31**	**0.13**	**Repelled by M *lissogaster***
M	*lissogaster*	M	*cephi*	11	13	1	0.2	0.65	
M	***lissogaster***	**F**	***cephi***	**7**	**17**	**1**	**4.04**	**0.04**	**Attracted to M *lissogaster***

Table 2. Isolated insect mating trials matrix for different combination of *B. cephi* and *B. lissogaster* males and females. Mating attempts (physical contact, mounting by male), mating-type behavior (wing fanning, accelerated activity level and rapid antenna tapping in proximity of female), and copulation (successful insemination of female) form the response categories.

Female *cephi* x Male *cephi*				
Date	# of pairs	behavior	attempts	copulation
1/27/2012	5	1	1	1
1/30/2012	5	0	2	3
1/31/2012	5	0	1	2
2/1/2012	5	1	1	0
3/7/2012	2	0	0	1
3/8/2012	3	0	0	3
2/9/2012	2	0	0	0
3/12/2012	4	0	1	1
3/16/2012	5	0	1	1
Total	**36**	**2**	**7**	**12**

Female *lissogaster* x Male *lissogaster*				
Date	# of pairs	behavior	attempts	copulation
1/30/2012	5	1	1	0
1/31/2012	3	0	1	0
2/3/2012	9	0	4	0
3/7/2012	1	0	1	0
3/12/2012	1	0	1	0
3/15/2012	4	0	0	2
3/21/2012	7	0	2	0
3/23/2012	5	0	1	1
3/27/2012	3	0	1	1
Total	**38**	**1**	**12**	**4**

Female *cephi* x Male *lissogaster*				
Date	# of pairs	behavior	attempts	copulation
2/6/2012	5	0	0	0
3/9/2012	1	0	0	0
3/12/2012	1	0	0	0
3/14/2012	1	0	0	0
3/16/2012	3	0	0	0
3/28/2012	3	0	0	0
4/3/2012	2	0	0	0
Total	**16**	**0**	**0**	**0**

Female *lissogaster* x Male *cephi*				
Date	# of pairs	behavior	attempts	copulation
3/7/2012	3	0	1	0
3/9/2012	1	0	0	0
3/14/2012	5	0	0	0
3/28/2012	4	0	0	0
Total	**13**	**0**	**1**	**0**

DISCUSSION: FIELD POPULATION ABUNDANCE SURVEY, SUMMER 2010-2011

To date, this temporal sampling program is the most comprehensive for populations of braconid parasitoids of *C. cinctus* in Montana. For each field site, 20 samples comprised of approximately 2,000 sweeps along 1,640 m of transects were taken weekly. The results indicate that not only do the populations of *B. cephi* and *B. lissogaster* overlap temporally, but their initial emergence occurs within days of each other, with populations of both species peaking during the same weeks in the summers of 2010 and 2011 for certain fields. Under continuous laboratory rearing, unlike in the field, parasitoid emergence from wheat residue taken from the same locations where both species were present revealed a different emergence sequence: *B. cephi* males, followed by *B. cephi* females, then a short period of several days of no new emergences, followed by the emergence of *B. lissogaster* males, then *B. lissogaster* females.

Also of note are the differences in parasitoid abundance between fields located in relatively close proximity to each other. The two Conrad field sites from 2010 and 2011 were within 10 km of each other, yet displayed different peak numbers and ratios of the two parasitoid species. Differences in field management and agronomic practices between growers are likely responsible for some of this variation, but the regional distribution of these flying insects remains difficult to characterize.

This is the first study to attempt to find a link between adult parasitoid abundance and the percent of *C. cinctus* infested wheat stems that were subsequently parasitized. The goal for this facet of the study was to establish a link between the number of female

parasitoids collected within specific areas (in this case, 16 different 20 x 20 m squares per field), and the subsequent rates of parasitism. Unfortunately, the data do not support this conclusion, with no significant correlations between parasitism rates and the number of female parasitoids for any field either year. Even though the correlation between parasitism and host infestation clustering patterns is well documented for within-stem populations of these braconids (Weaver *et al*., 2005), there is very little known about the distributions and aggregations of flying adults during mating and oviposition. Any further investigation into the relationship between parasitism rates and numbers of flying females would benefit from a characterization of the in-field aggregations and distributions of adults, perhaps through a mark and recapture program.

DISCUSSION: DUFOUR'S GLAND CHEMICAL ANALYSIS

The vast majority of effort involved in investigating the acetate esters identified by Baker et al. (2005) in the Dufour's glands of female *B. cephi* and *B. lissogaster* was directed towards the development of methods to allow the quantification of these acetate esters. Qualitative analysis of the acetate esters by comparing percentages of the total ion count allows for quick comparisons, but is not conducive to the calculation of absolute concentrations and amounts of material. A quantitative approach is valuable in elucidating the exact differences in the chemical composition of the gland contents of the two species. A solution chemistry approach was required to accurately assess the ratios and concentrations of the acetate esters of interest in the Dufour's glands of the two species. Diluting the contents of a single Dufour's gland 0.5-0.9 mm long into a volume large enough to be sampled and injected into a GC/MS resulted in chromatographic traces at the limit of detector sensitivity. Baseline obfuscation rendered the identification of mass spectra problematic and necessitated a change in methodology to boost the concentration of the gland contents being sampled. Unfortunately, adding more glands to the solution had undesirable side effects that concentrate long-chain fatty acids which makes chromatography impossible.

As a result, a new technique was devised that involved piercing glands with glass needles, then immersing the glands into hexane for several seconds before removing them. The subsequent solution was then decanted to minimize the possibility of tissue transfer using the low vapor pressure of hexane to form a pressure gradient from a lightly sealed container into an open one. Glass capillary tubing and air-tight rubber septa were

used as the conduits for this solution decanting. Once transferred, the solution was then evaporated under nitrogen to 25% of its original volume. Four of these one-gland decanted solutions were then combined using the same lightly pressurized capillary tubing system into a volume large enough to be handled by the auto-injector on the GC/MS. This method ensured reasonably defined chromatography while minimizing the risks associated with foreign-matter introduction to a gas column.

However, variation in the quality of field collected and newly emerged insects used for Dufour's gland analysis resulted in inconsistent gland dissections and extractions. This likely contributed to the difficulty in getting similar peak areas for identical compounds from different solutions. As a result, representative solutions for *B. cephi* and *B. lissogaster* that provided the cleanest chromatography and similar peak area sizes for shared compounds was selected to be the "type" Dufour's gland signature for their respective species. As the solution contains the glandular extracts from four different individuals, there is an averaging effect. However, between-gland variation was not possible to characterize.

The use of response curves from synthetic acetate ester standards to integrate Dufour's gland peak areas allowed for an accurate assessment of the quantities and ratios of each acetate ester and indicates that substantial differences exist between the two species. Female *B. cephi* and *B. lissogaster* Dufour's glands shared three acetate esters between them (*n*-tetradecyl acetate, (*Z*)-7-hexadecen-1-yl acetate, and (*Z*)-9-hexadecen-1-yl acetate), but in differing amounts. Interestingly, for both species, (*Z*)-7-hexadecen-1-yl acetate was found in the greatest amount, followed by *n*-tetradecyl acetate. Both

species had similar amounts of (*Z*)-9-hexadecen-1-yl acetate, but (*Z*)-11-hexadecen-1-yl acetate was only detected in *B. lissogaster*. If these acetate esters somehow play a role in maintaining reproductive isolation between these two species, it is most probable that they work in concert with each other in a balanced olfactory antagonism (Baker 2008). This idea posits that pheromone components must be present in a certain ratio to each other to achieve an attractive effect because slight changes in this ratio can result in behavioral antagonism, with no effect or even a repellent effect on potential mates. In addition, it is well documented that the interactions of insect pheromones and plant semiochemicals are often critical to the effective functioning of aggregation pheromones in many insect species (Reddy and Guerrero 2004), indicating that the quantification of acetate esters in the Dufour's glands of these two parasitoid species may only be the first step in exploring the composition of mating pheromones in these two species.

DISCUSSION: ELECTROPHYSIOLOGY OF CANDIDATE SEX PHEREMONES

An electrophysiological examination made of male *B. cephi* and *B. lissogaster* antennae using the four isolated acetate ester components from the Dufour's glands of the females of both species indicated that by themselves, these acetate esters produce no significant difference in the response of male antennae to dramatically different dose amounts or type of acetate ester. Increasing variability in the scale of depolarizations as a function of time for each assay meant that it became more difficult to characterize subtle changes in electrical activity as the lifetime of the antennal preparation wore on. The mean depolarization values for *B. cephi* and *B. lissogaster* suggest a possible increase in electrical activity as the dose of each acetate ester increased, the dramatic fluctuations in the responses to the standard controls were also found in the responses to acetate esters over time.

To view how a representative depolarization might look when an antenna was exposed to another potential attractant, two plant volatiles released by wheat plants during injury by wheat stem sawfly larvae known to be strong attractants to female *B. cephi* and *B. lissogaster* (Perez 2009) were also tested using electroantennography. The semiochemicals, 6-methyl-5-hepten-2-one and (*E*)-2-hexanyl acetate, both produced depolarizations in *B. cephi* and *B. lissogaster* male antennae that were substantially greater than depolarizations produced by any of the acetate esters or hexane controls, despite the fact that Perez (2009) did not find these to be significantly active for these males. Because one would expect sex pheromone components to produce

electrophysiological depolarizations similar or greater than responses elicited by host location cues, this sensory level data demonstrate that the individual acetate esters examined play a negligible role in regulating mating of *B. cephi* and *B. lissogaster*.

DISCUSSION: BEHAVIORAL ASSAYS OF LIVE INSECTS

The results of the Y-tube behavioral assays suggest significant preference over the control for female *B. cephi* by female *B. lissogaster* responders, and for male *B. lissogaster* by male *B. lissogaster* and female *B. cephi* responders. In addition, male *B. cephi* and *B. lissogaster* may have preferences for the females of the same species over the controls, but the data are not conclusive. This may be due to the fact that *B. cephi* is known to have a pre-oviposition period of several weeks after eclosion, but *B. lissogaster* has none (Holmes 1963). Mating trials indicate that the rare cross-species preferences shown in the Y-tube assays do not translate to mating attempts or mating-type behavior in any cross-species pairings, but that same-species pairings do translate to mating attempts, copulation, and mating-type behavior for close to 50% of the replications.

This suggests that either the methods used in the Y-tube assays did not account for all of the possible variables that could have affected the choices made by responding insects, like the need for *B. cephi* to have a protracted pre-oviposition period, or that additional species isolating mechanisms are in effect when insects are within close proximity to one another. One would expect that the same-species close-range attractions that result in mating attempts and copulation would also be plainly evident in Y-tube assay results; yet this was not consistent. Similarly, the same lack of attraction and mating attempts in cross-species pairings would also be expected to be observed in Y-tube assays exhibiting the same pairings. However, the significant preference for the cross-species odor sources in two Y-tube pairings confounds these expectations and may indicate additional complexity in the chemical ecology of these two insects.

Male genitalic characters are often used to differentiate insect taxa, and are highly conserved at the genus and species levels in many insect orders (Matsuda, 1976). A study of several genera of the subfamily Agathidinae (Hymenoptera: Braconidae) by Brajkovic et al. (2010) indicated that male genitalic characters can be used to differentiate between genera, but that differentiation of species within genera is difficult unless the genus contains enough species to be diagnostic and other external characters are used as well. The similarities encountered in the genitalia of congeneric species make differentiation rely on subtle changes in the pilosity and curvature of the clasping surfaces of the aedeagus. Future comparative study of the aedeagii of *B. cephi* and *B. lissogaster,* as well as other members in the genus, may better establish the existence of a physical barrier to copulation between the two species. Although the behavior of both species in close confinement with each other does not indicate that differences in reproductive anatomy are being used as the primary mechanism for reproductive isolation, the existence of such a barrier may give insight into the role that sex pheromones have played in the evolution of these two species.

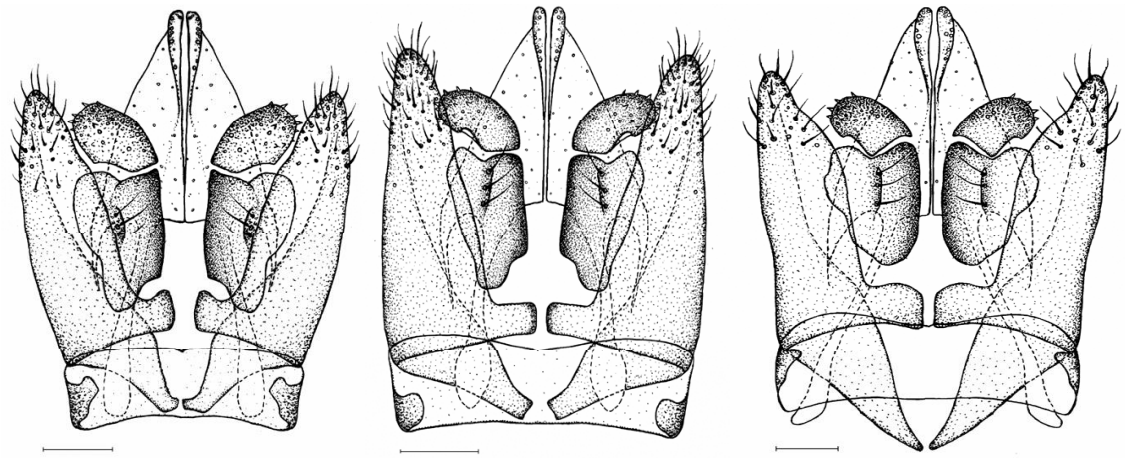


Figure 12. Male genitalia of *Cremnops vulgaris* (Cresson 1865) (left), *Cremnops haematodes* (Brull1846) (center) and *Cremnops montrealensis* (Morrison 1917) (right). Scale bar 0.1 mm. (From Brajkovic et al. 2010).

CONCLUSION

The presence of two sympatric and temporally concurrent congeneric braconid parasitoids of *C. cinctus* in wheat fields in Montana suggests that a mechanism exists for the maintenance of reproductive isolation between these two species. Isolation and quantification of a group of acetate esters in these two species confirmed the findings of Baker *et al.* (2005) and revealed the specific ratios of these acetate esters to each other in the Dufour's glands of *B. cephi* and *B. lissogaster*. However, although previously suggested to have possible behavioral activity, electrophysiological assays of these individual acetate esters suggest that little sensory activity can be attributed to them. This does not rule out the possibility of a sex pheromone that uses these acetate esters in conjunction with other insect or plant-generated volatiles, but it has been demonstrated that by themselves, they elicit minimal responses. Y-tube behavioral assays of both species indicate attraction towards members of the opposite sex, as well as other more anomalous preferences. Further method development may be necessary to eliminate potential sources of variance; for example, correction for dramatic changes in barometric pressure increased the stability of the responses, but the results did not match what one would expect given the clear mating preferences and activities exhibited in the mating trials. More avenues for research regarding behavioral interactions between *B. cephi* and *B. lissogaster* as well as examination of male genitalic characters would likely be useful in elucidating potential isolating mechanisms and ultimately lead to better management practices for populations of these endemic parasitoids in Montana wheat fields.

LITERATURE CITED

Ainslie, C.N. (1920). The western grass-stem sawfly. *U.S. Department of Agriculture Bulletin* **841**. Washington, D.C.

Ali, F.A. and Morgan, E.D. (1990). Chemical communication in insect communities: a guide to insect pheromones with a special emphasis on social insects. *Biological Reviews* **65**: 227-247.

Anonymous (1997). Wheat stem sawfly. Montana Crop Health Report: Special Edition. *Montana Extension Service* **10**: 1-7.

Anonymous (1999). Heptachlor. http://www.epa.gov/epawaste/hazard/wastemin/minimize/factshts/hepchl.pdf Accessed 11-23-2012.

Baker, J., Howard, R., Morrill, W., Meers, S., Weaver, D. (2005). Acetate esters of saturated and unsaturated alcohols (C12-C20) are major components in Dufour glands of *Bracon cephi* and *Bracon lissogaster (*Hymenoptera: Braconidae), parasitoids of the wheat stem sawfly (Hymenoptera: Cephidae). *Biochemical Systematics and Ecology* **33**: 757-769.

Baker, Thomas C. (2008). Balanced olfactory antagonism as a concept for understanding evolutionary shifts in moth sex pheromone blends. *Journal of Chemical Ecology* **34**: 971-981

Bartlet, R. J, Cossé A. A., Petroski, R. J., and Weaver, D. K. (2002). Cuticular hydrocarbons and novel alkenediol diacetates from wheat stem sawfly (*Cephus cinctus*): natural oxidation to pheromone components. *Journal of Chemical Ecology* **28**:385-405.

Beirne, B. P. (1972). The biological control attempt against the European wheat stem sawfly, *Cephus pygmaeus* (Hymenoptera: Cephidae), in Ontario. *Canadian Entomologist* **104**: 987-990.

Beres, B. L., Carcamo, H.A., and Byers, J.R. (2007). Effect of wheat stem sawfly damage on yield and quality of selected Canadian spring wheat. Journal of Economic Entomology, **100**: 79-87.

Beres, B.L., Carcamo, H.A., and Bremer, E. (2009). Evaluation of alternative planting strategies to reduce wheat stem sawfly (Hymenoptera: Cephidae) damage to spring wheat in the Northern Great Plains. Journal of Economic Entomology, **102**: 2137-2145.

Beres, B. L., Carcamo, H.A., Dosdall, L.M., Yang, R., Evenden, M.L., and Spander, D.M. (2011). Do interactions between residue management and direct seeding system affect wheat stem sawfly and grain yield? *Agronomy Journal* **103**: 1635-1644.

Beres, B.L., Carcamo, H.A., Weaver, D.K., Dosdall, L.M., Evenden, M.L., Hill, B.D., Yang, R.C., McKenzie, R.H., and Spaner, D.M. (2011b). Integrating the building blocks of agronomy and biocontrol into an IPM strategy for wheat stem sawfly. *Prarie Soils and Crops* **4:** 54-65.

Blodgett, S. L., H. B. Goosey, D. K. Waters, C. I. Tharp, and G. D. Johnson. (1996a). Wheat stem sawfly control on winter wheat. *Arthropod Management Tests* **22**: 331-332.

Blodgett, S. L., G. D. Johnson, W. T. Lanier, H. B. Goosey, A. W. Lenssen, D. K. Waters, Talbert, L. E., and P. L. Bruckner. (1996b). Special issue: Wheat stem sawfly. *Montana Crop Health Report* **9**: 1-7.

Bowden, R., Shoryer, J., Roozeboom, K., Claassen, M., Evans, P., Gordon, B., Heer, B., Janssen, K., Long, J., Martin, J., and others (2001). Performance of wheat variety blends in Kansas. *In* Keeping up with research. Kansas State University, Manhattan, KS. p. 6.

Brajkovich, M., Stanisavljevic, Z., Nikolic, S., Curcic, S. B., Zivic, I., and Stojanovic, D. (2010). Male genitalia of the species of the subfamily agathidinae (Hymenoptera: Braconidae) and their importance in taxonomy. *Archives of Biological Sciences, Belgrade* 62: **455-467**

Brandt, S.A., Kutcher, H. R., and Smith, E.G. (2008). Agronomic implications of more intensive canola rotations. *Proceedings of FARMTECH 2008* **136**.

Butcher, F. G. (1946). The wheat stem sawfly. *North Dakota Agriculture Col. Extension Service Cir.* **A-94**. 5 p.

Buteler, M., Weaver, D.K., and Miller, P.R. (2008). Wheat stem sawfly-infested plants benefit from parasitism of the herbivorous larvae. Agricultural and Forest Entomology, **10**: 347-354; *Corrigendum* 11:123.

Callenbach, J. A. and Hansmeier, M. P. (1944). Wheat stem sawfly control in severely infested areas. *Montana Extension Service in Agriculture and Home Economics Circular* **156**.

Callenbach, J. A. and Hansmeier, M. P. (1945). Wheat stem sawfly control. *Montana Extension Service in Agriculture and Home Economics Circular* **164**.

Carcamo, H.A., Beres, B.L., Clarke, F., Byers, R.J., Mundel, H.H., May, K. and DePauw, R. (2005). Influence of plant host quality on fitness and sex ratio of the wheat stem sawfly (Hymenoptera: Cephidae). Environmental Entomology, **34**: 1579-1592.

Carcamo, H.A., and Beres, B.L. (2006). Does wheat host affect overwintering survivorship of the wheat stem sawfly? Proceedings of the Annual Meeting of the Entomological Society of Alberta, **54**: 1.

Carpenter, J. E., and Jewett, D. K. (2003). Influence of herbivore-damaged corn and cotton in the field recruitment of braconids parasitoids from feral populations. *Florida Entomologist* **86**: 233-238.

Chen, L., and Fadamiro, H. Y. (2007). Differential electroantennogram response of females and males of two parasitoid species to host-related green leaf volatiles and inducible compounds. *Bulletin of Entomological Research* **97**: 515-522.

Church, N. S. (1955). Moisture and diapause in the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). *Canadian Entomologist* **87**: 85-87.

Cochran, V., Danielson, J., Kolberg R., and Miller, P. (2006). Dryland cropping in the Canadian prairies and the US Great Plains. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. Dryland Agriculture, 2nd ed., Agronomy Monograph **23**.

Conti, E., G. Salerno, F. Bin, and S. B. Vinson (2003). The role of host semiochemicals in parasitoid specificity: a case study with *Trissolcus brochymenae* and *Trissolcus simony* on pentatomid bugs. *Biological Control* **29**: 435-444.

Cortesero, A. M., C. M. De Moraes, J. O. Stapel, J. H. Tumlinson, and W. J. Lewis (1997). Comparisons and contrasts in host foraging strategies of two larval parasitoids with different degrees of host specificity. *Journal of Chemical Ecology* **23**: 1589-1606.

Cossé, A. A., R. J. Bartlet, D.K. Weaver, and B. W. Zilkowski (2002). Pheromone components of the wheat stem sawfly: identification, electrophysiology and field bioassay. *Journal of Chemical Ecology* **28**: 407-423.

Criddle, N. (1922a). The western-stem sawfly and its control. Canadian. *Department of Agriculture* Pamphlet No. **6**.

Criddle, N. (1922b). The western wheat stem sawfly in Canada. The Report of the Entomological Society No. **36**: 18-21

Davis, E. G. (1955). Status of the wheat stem sawfly in the United States in 1954. *North Dakota Agriculture Experiment Station* **17**: 171-175.

Davis, E. G., C. Benton, and H. W. Somsen (1955). Natural enemies of the wheat stem sawfly in North Dakota and Montana. *North Dakota Agriculture Experiment Station* Bulletin **18**: 63-65.

Delaney, K.J., Weaver, D.K., and Peterson, R.K.D. (2010). Photosynthesis and yield reductions from wheat stem sawfly (Hymenoptera: Cephidae): Interactions with wheat solidness, water stress, and phosphorus deficiency. Journal of Economic Entomology, **103**: 516-524.

Drost, Y. C., W. J. Lewis, and J. H. Tumlinson (1988). Beneficial Arthropod behavior mediated by airborne semiochemicals V. Influence of rearing method, host plant, and adult experience an host-searching behavior of *Microplitis croceipes* (Cresson), a larval parasitoid of *Heliothis.* Journal of Chemical Ecology **14**: 1607-1616.

Eller, F. J., J. H. Tumlinson, and W. J. Lewis (1988a). Beneficial arthropod behavior mediated by airborne semiochemicals: source of volatiles mediating the host-location flight behavior of *Microplitis croceipes* (Cresson)(Hymenoptera: Braconidae), a parasitoid of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae). Environmental Entomology **17**: 745-753.

Eller, F. J., J. H. Tumlinson, and W. J. Lewis (1988b). Beneficial arthropod behavior mediated by airborne semiochemicals II: Olfatometric studies of host location by the parasitoid *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae). Journal of Chemical Ecology **14**: 425-434.

Eller, F. J., J. H. Tumlinson, and W. J. Lewis (1992). Effect of host diet and preflight experience on the flight responses of *Microplitis croceipes* (Cresson). *Physiological Entomology* **17**: 235-240.

Farstad, C. (1940). The development of western wheat stem sawfly (*Cephus cinctus* Nort.) in various host plants as an index of resistance [PhD dissertation]. Iowa State College, Ames, IA. 83 p.

Farstad, C.W., King, K.M., Glen, R., and Jacobsen, L.A. (1945). Control of the wheat stem sawfly in the prairie provinces. War-time Production series- Agricultural Supplies Board Special Publication **59**: 1-7.

Fatouros, N. E, M. Dicke, R. Mumm, T. Meiners, and M. Hilker (2008). Foraging behavior of egg parasitoids exploiting chemical information. Behavioral Ecology **19**: 677-689.

Foster, S. P. and M. O. Harris (1997). Behavioral manipulation methods for insect pest-management. *Annual Review of Entomology* **42**: 123-146.

Fritzsche, M. E. and C. J. Turlings (2001). Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids. *Evolutionary Ecology Research* **3**: 553-565.

Gahan, A. B. (1918). Description of a new hymenopterous parasite (Braconidae). *Proceedings of the Entomological Society of Washington* **20**: 18-19.

Gall, A. and J. R. Dogger (1967). Effect of 2,4-D on the wheat stem sawfly. *Journal of Economic. Entomology* **60***: 75-77.*

Goosey, H. B. (1999). In field distribution of the wheat stem sawfly, (Hymenoptera: Cephidae), and evaluations of selected tactics for an integrated management program. [Master‟s Thesis]. Montana State University, Bozeman, Montana. 83 p.

Hérard, F., M. A. Keller, W. J. Lewis and J. H. Tumlinson (1988). Beneficial arthropod behavior mediated by airborne semiochemicals III. Influence of age and experience on flight chamber responses of *Microplitis demolitor* Wilkinson. *Journal of Chemical Ecology* **14**: 1583-1596.

Hérard, F., M. A. Keller, W. J. Lewis and J. H. Tumlinson (1988). Beneficial arthropod behavior mediated by airborne semiochemicals IV. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson. *Journal of Chemical Ecology* **14**: 1597-1605.

Holmes, N. D. (1953). Note on *Scambus detritus* (Holmg) (Hymenoptera: Ichneumonidae), a new parasite of wheat stem sawfly, *Cephus cinctus North Canadian Entomologist* **85**: 339.

Holmes, N. D. (1954). Food relations of the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae). *Canadian Entomologist* **86**: 159-167.

Holmes, N. D. and C. W. Farstad (1956). Effects of field exposure on immature stages of the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae). *Canadian Journal of Agricultural Science* **36**: 196-202.

Holmes, N. D. and L. K. Peterson (1960). The influence of the host on oviposition by the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae). *Canadian Journal of Plant Science* **40**: 29-46.

Holmes, N. D. and L. K. Peterson (1962). Resistance of spring wheats to the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae) II. Resistance to the larva. *Canadian Entomologist* **94**: 348-365.

Holmes, N. D. and L. K. Peterson (1963). Heptachlor as a systemic insecticide against the wheat stem sawfly, *Cephus cinctus. Northern Canadian Entomologist* **95**: 792-796.

Holmes, N. D., W. A. Nelson, L. K. Peterson, and C. W. Farstad (1963). Causes of variation in effectiveness of *Bracon cephi* (Gahan) (Hymenoptera: Braconidae) as a parasite of the wheat stem sawfly. *Canadian Entomologist* **95**: 113-126.

Holmes, N. D. (1977). The effect of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), on the yield and quality of wheat. *Canadian Entomologist* **109**: 1591-1598.

Holmes, N. D. (1979). The wheat stem sawfly. *Proceedings of the 26th Annual Meeting of the Entomological Society of Alberta* **26**: 2-13.

Holmes, N. D. (1982). Population dynamics of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), in wheat. *Canadian Entomologist* **114**: 775-788.

Ivie, M. A. (2001). On the geographic origin of the wheat stem sawfly (Hymenoptera: Cephidae): a new hypothesis of introduction from northeastern Asia. *American Entomologist* **47**: 84-97.

Ivie, M. A. and A. G. Zinovjev (1996). Discovery of the wheat stem sawfly (*Cephus cinctus* Norton) (Hymenopter: Cephidae) in Asia, with the proposal of a new synonymy. *Canadian Entomologist* **128**: 347-348.

Jacobson, L. A. and C. W. Farstad (1952). Effect of time of seeding apex wheat on infestation and sex ratio of the wheat stem sawfly, *Cephus cinctus* Norton. (Hymenoptera: Cephidae). *Canadian Entomologist* **84**: 90-92.

Kemp, H. L. 1934. Studies on solid stem wheat varieties in relation to wheat stem sawfly control. *Scientia Agricola* **15**: 30-38.

Khan, Z. R., J. A. Pickett, J. van den Berg, L. J Wadhams, and C. M. Woodcock (2000). Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa. *Pest Management Science* **56**:957-962.

Knodel, J.J., Beauzay, P.B., Eriksmoen, E.D., and Pederson, J.D. (2009). Pest management of wheat stem maggot (Diptera: Chloropidae) and wheat stem sawfly (Hymenoptera: Cephidae) using insecticides in spring wheat. *Journal of Agricultural and Urban Entomology* **26**: 183-197.

Lanning, S.P., Carlson, G.R., Nash, D., Wichman, D.M., Kephart, K.D., Stougaard, R.N., Kushnak, G.D., Ecknoff, J.L., Grey, W.E., Talbert, L.E. (2004). Registration of 'Choteau' wheat. (Registrations of Cultivars). *Crop Science*. Retrieved March 12, 2013 from HighBeam Research: http://www.highbeam.com/doc/1G1-126074318.html

Luginbill, P. (1958). Influence of seeding density and row spacing on the resistance of spring wheat to the wheat stem sawfly. *Journal of Economic Entomology* **51**: 804-808.

Luginbill, P. and E. F. Knipling (1969). Suppression of wheat stem sawfly with resistant wheat. *ARS-USDA, Production Research Report* No. **107**, 9 p.

Macedo, T.B., Weaver, D.K., and Peterson, R.K.D. (2007). Photosynthesis in wheat at the grain filling stage is altered by larval wheat stem sawfly (Hymenoptera: Cephidae) injury and reduced water availability. Journal of Entomological Science, **42**L 228-238.

Mantel, Nathan (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**: 209

Marris, G.C., Hubbard, S.F. Scrimgeour, C. (1996). The perception of genetic similarity by the solitary parthenogenic parasitoid *Venturia canescens*, and its effects on the occurrence of superparasitism. *Entomologia Experimentalis et Applicata* **78**, 176-174.

Matsuda, R. (1976). Morphology and Evolution of the Insect Abdomen. Pergamon Press, New York, 534 pp.

McBride, D. K., D. D. Koop, and C. W. Nyegaard (1996). Wheat stem insect pests and management practices. *North Dakota State University Extension Service, Fargo, ND* **E-680**.

McNeal, F. H., M. A, Berg, and P. Luginbill (1955). Wheat stem sawfly damage in four spring wheat varieties as influenced by date of seeding. *Agronomy Journal* **47**: 522-525.

Meers, S. B. (2005). Impact of harvest operations on parasitism of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). Master's Thesis. Entomology, Montana State University, Bozeman, Montana. 129 p.

Montana State University Agricultural Experiment Station (2011). 2011 spring wheat varieties: performance evaluations and recommendations. Accessed 7-5-2012. http://plantsciences.montana.edu/crops/springwheat/2011data/2011SpringWheatVarieties.pdf

Morgan, E. David (1990). Preparation of small-scale samples from insects for chromatography. *Analytica Chimica Acta* **236**: 227-235.

Morrill, W. L., J. W. Gabor, and G. D. Kushnak (1992). Wheat stem sawfly (Hymenoptera: Cephidae) damage and detection. *Journal of Economic Entomology* **85**: 2413-2417.

Morrill, W. L., J. W. Gabor, and D. Wichman (1993). Mortality of the wheat stem sawfly (Hymenoptera: Cephidae) at low temperatures. *Environmental Entomology* **22**: 1358-1361.

Morrill, W. L., G. D. Kushnak, P. L. Bruckner, and J. W. Gabor (1994). Wheat stem sawfly (Hymenoptera: Cephidae) damage, rates of parasitism, and overwinter survival in resistant wheat lines. *Journal of Economic Entomology* **87**: 1373-1376.

Morrill, W. L. and G. D. Kushnak (1996). Wheat stem sawfly (Hymenoptera: Cephidae) adaptation to winter wheat. *Environmental Entomology* **25**: 1128-1132.

Morrill, W. L. (1997). The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), and associated parasitoids in the northern Great Plains of North America. Trends in Entomology **1**: 171-174.

Morrill, W. L., G. D. Kushnak, and J. W. Gabor (1998). Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) in Montana. *Biological Control* **12**: 159-163.

Morrill, W. L. and G. D. Kushnak. 1999. Planting date influence on the wheat stem sawfly (Hymenoptera: Cephidae) in spring wheat. *Journal of Agricultural Urban Entomology* **16**: 123-128.

Morrill, W. L., J. W. Gabor, D. K. Weaver, G. D. Kushnak, and N. J. Irish (2000). Effect of host plant quality on the sex ratio and fitness of female wheat stem sawflies (Hymenoptera: Cephidae). *Environmental Entomology* **29**: 195-199.

Morrill, W. L., D. K. Weaver, and G. D. Johnson (2001a). Trap strip and field modification for management of the wheat stem sawfly (Hymenoptera: Cephidae). *Journal of Entomological Science* **36**: 34-45.

Morrill, W. L., D. K. Weaver, N. J. Irish, and W. F. Barr (2001b). *Phyllobaenus dubius* (Wolcott) (Coleoptera: Cleridae), a new record of a predator of the wheat stem sawfly (Hymenoptera: Cephidae). *Journal of the Kansas Entomological Society* **74**: 181-183.

Mudd, A., Fisher, R.C., Smith, M.C. (1982). Volatile hydrocarbons in the Dufour's gland of the parasite *Nemeritis canescens* Grav. (Hymenoptera: Ichneumonidae). Journal of Chemical. Ecology **8**: 1035-1042.

Munro, A. J. (1947). Wheat stem sawfly is on the wing. *National Federation of Grain Cooperatives Coop Grain Quarterly* **5(3)**: 39-42.

Munro, J. A., W. Nostdahl, and R. L. Post (1949). Wheat stem sawfly. *North Dakota Agriculture Collective Bimonthly Bulletin* **11**: 85-91.

Nansen, C., T. B. Macedo, D. K. Weaver, and R. K. D. Peterson (2005a). Spatiotemporal distributions of wheat stem sawfly eggs and larvae in dryland wheat fields. *Canadian Entomologist* **137**: 428-440.

Nansen, C., D. K. Weaver, S. E. Sing, J. B. Runyon, W. L. Morrill, M. J. Grieshop, C. L. Shannon, Johnson, M.L. (2005b). Within-field spatial distribution of *Cephus cinctus* (Hymenoptera: Cephidae) larvae in Montana wheat fields. *Canadian Entomologist* **137**: 202-214.

Nelson, W. A. and C. W. Farstad (1953). Biology of *Bracon cephi* (Gahan) (Hymenoptera: Braconidae), an important native parasite of the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae), in Western Canada. *Canadian Entomologist* **85**: 103-107.

Noldus, L. P. J. J. (1988). Response of the egg parasitoid *Trichogramma pretiosum* to the sex pheromone of its host *Heliothis zea*. *Entomoligia Experimentalis et Applicata* **48**: 293-300.

Noldus, L. P. J. J., W. J. Lewis and J. H. Tumlinson (1990). Beneficial arthropod behavior mediated by airborne semiochemicals. IX. Differential response of *Trichogramma pretiosum,* an egg parasitoid of *Heliothis zea,* to various olfactory cues. *Journal of Chemical Ecology* **16**: 3531-3544.

Norton, E. (1872). Notes on North American Tenthredinidae with descriptions of new species. *Transactions of the American Entomological Society* **4**: 77-86.

Perez-Medoza, J., D. K. Weaver, and W. L. Morrill (2006). Infestation of wheat and downy brome grass by wheat stem sawfly and subsequent larval performance. *Environmental Entomology*. **35**: 1279-1285.

Perez, O. G. (2009). Behavioral and sensory responses of endemic braconid parasitoids to changes in volatile emissons induced by wheat stem sawfly, *Cephus cinctus*, herbivory. Doctoral Dissertation. Montana State University, Bozeman, MT.

Pickett, J. A., L. J. Wadhams, and C. M. Woodcock (1997). Developing sustainable pest control from chemical ecology. *Agricultural Ecosystems and Environment* **64**: 149-156.

Platt, A. W., C. W. Farstad, and J. A. Callenbach (1948). The reaction of Rescue wheat to sawfly damage. *Scientia Agricola* **28:** 154-161.

Quisenberry, S. S., D. J. Schotzko, P. F. Lamb, and F. L. Young (2000). Insect distribution in spring pea-winter wheat-spring barley crop rotation system. *Journal of Entomological Science* **35**: 327-333.

Reddy, Gadi V. P. and Guerrero, Angel (2004). Interactions of insect pheromones and plant semiochemicals. *Trends in Plant Science* **9**:253-261.

Riley, C. V. and C. L. Marlatt (1891). Wheat and grass-sawflies. *Insect Life* **4**: 168-179.

Roemhild, G. R. (1954). Morphological resistance of some of the Gramineae to the wheat stem sawfly (*Cephus cinctus* Norton) [Master‟s Thesis]. Montana State College, Bozeman, MT. 42 p.

Runyon, J.B. (2001). Wheat stem sawfly parasitism in varying field sizes and tillage systems in dryland wheat in Montana. Master's Thesis. Department of Entomology, Montana State University, Bozeman, Montana. 75 pp.

Runyon, J.B., Hurley, R.L., Morrill, W.L., Weaver, D.K. (2001). Distinguishing adults of *Bracon cephi* and *Bracon lissogaster* (Hymenoptera: Braconidae), parasitoids of the wheat stem sawfly (Hymenoptera: Cephidae). *Canadian Entomologist* **133**: 215-217.

Runyon, J. B., W. L. Morrill, D. K. Weaver, and P. R. Miller (2002). Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) by *Bracon cephi* and *B, lissogaster* (Hymenoptera: Braconidae) in wheat fields bordering tilled and untilled fallow. *Montana Journal of Economic Entomology* **95**: 1130-1134.

Sabelis, M. W., A. Janssen, and M. R. Kant (2001). Enhanced: the enemy of my enemy is my ally. *Science* **291**: 2104-2105.

Saint Pierre, C., Trethowan, R., and Reynolds, M. (2010). Stem solidness and its relationship to water-soluble carbohydrates: association with wheat yield under water deficit. Functional Plant Biology, **37**: 166-174.

Salt, R. W. (1946). Moisture relationships of the wheat stem sawfly (*Cephus cinctus* Norton) I. Some effects of desiccation. *Scientia Agricola* **26**: 622-630.

Salt, R. W. (1961). Principles of insect cold-hardiness. *Annual Review of Entomology* **6**: 55-74.

Seamans, H. L. G.F. Manson, and C.W. Farstad (1938). The effect of the wheat stem sawfly (*Cephis cinctus* Norton) on the heads and greain of infested stems. *Annual Report of the Entomological Society of Ontario* **75:** 10-15.

Seamans, H. L., G. F. Manson, and C. W. Farstad (1944). The effect of the wheat stem sawfly (*Cephus cinctus* Nort.) on the heads and grains of infested stems. *Ontario Entomological Society Annual Report* **75**: 10-15.

Smith, C. W. (1931). Colonization in Canada of *Collyria calcitrator* (Hymenoptera.: Ichneumonidae.), a parasite of the wheat stem sawfly. *Bulletin of Entomological Research* **22**: 547-550.

Smith, R. W. (1959). Status in Ontario of *Collyria calcitrator* (Grav.) (Hymenoptera: Ichneumonidae) and *Pediobius beneficus* (Gahan) (Hymenoptera: Eulophidae) as parasites of the European wheat stem sawfly, *Cephus pygmaeus* (L.) (Hymenoptera: Cephidae). *Canadian Entomologist* **91**: 697-700.

Smith, R. W. (1961). Notes on parasites of the wheat stem sawfly, *Cephus pygmaeus* (L.) (Hymenoptera: Cephidae) from continental Europe. *Canadian Entomologist* **93**: 714-717.

Somsen, H. W. and P. Luginbill (1957). *Bracon lissogaster* Mues., a parasite of the wheat stem sawfly. *USDA Technical Bulletin* **1153**.

Streams, F. A. and L. W. Coles (1965). Wheat stem sawfly parasites, *Collyria calcitrator* and *Pediobius nigritarsis* in Eastern United States. *Journal of Economic Entomology* **58**: 303-306.

USDA National Agricultural Statistics Service (2011). 2011 State agriculture overview, Montana. Accessed 11-20-2012.

http://www.nass.usda.gov/Statistics_by_State/Ag_Overview/AgOverview_MT.pdf

Wallace, L. E. (1962). Field-plot tests of chemicals for wheat stem sawfly control. *Journal of Economic Entomology* **55**: 908-912.

Wallace, L. E., and F. H. McNeal. 1966. Stem sawflies of economic importance in grain crops in the United States. *USDA Technical Bulletin* **1350**: 1-50.

Wallace, L.E., and Butler, L.I. (1967). Residues of heptachlor and heptachlor epoxide in wheat treated to control the wheat stem sawfly. Journal of Economic Entomology, **55**: 908-912.

Weaver, D. K., C. Nansen, J. B. Runyon, S. E. Sing, and W. L. Morrill (2005). Spatial distributions of *Cephus cinctus* Norton (Hymenoptera: Cephidae) and its braconid parasitoids in Montana wheat fields. *Biological Control* **34**: 1-11.

Weiss, M. J. and W. L. Morrill (1992). Wheat stem sawfly (Hymenoptera: Cephidae) revisited. *American Entomologist* **38**: 241-245.

Wenda-Piesik, A., Z. Sun, W. E. Grey, D. K. Weaver, and W.L. Morrill (2009). Mycoses of wheat stem sawfly (Hymenoptera: Cephidae) larvae by *Fusarium* spp. isolates.