

BEHAVIORAL AND SENSORY RESPONSES OF ENDEMIC BRACONID
PARASITOIDS TO CHANGES IN VOLATILE EMISSIONS INDUCED
BY WHEAT STEM SAWFLY, *CEPHUS CINCTUS* HERBIVORY

by

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ABSTRACT

Bracon cephi (Gahan) and *Bracon lissogaster* Muesebeck are considered the most effective biological control agents of the wheat stem sawfly, *Cephus cinctus* Norton, probably the most serious wheat insect pest in the northern Great Plains of North America. The great difficulty in controlling this pest suggests an urgent need to improve our understanding of the chemical ecology of these parasitoids to enhance effective control of this pest.

Chemical ecology has been demonstrated to be an effective tool in the control of important pests. Plants produce volatile compounds as defenses against herbivory. These compounds play an important role in host selection by herbivores and their natural enemies. Studies revealed that wheat plants infested by *C. cinctus* produce different amounts of specific compounds compared to uninfested plants.

Synthetic compounds matching those produced by sawfly infested wheat plants were tested in three different concentrations against males and females *B. cephi* and *B. lissogaster* parasitoids using an electroantennogram system. Six compounds generated positive electrophysiological responses from the parasitoid antennae. Behavioral bioassays using the previous responsive compounds determined that three compounds were attractive to parasitoids of both sexes.

To better understand parasitoid host seeking, it was important to quantify and determine the spatial distribution of these behaviorally active compounds as they were emitted from wheat plants. Volatile compounds were collected from infested and uninfested wheat plants at three different heights and two distances in the greenhouse. Greater amounts were collected from the infested plants immediately adjacent to the base of the plants.

Field studies were performed to compare concentrations of volatile compounds between areas of heavy and light sawfly infestation. Greater amounts of behaviorally active volatile compounds were collected from areas of heavier sawfly infestation.

In conclusion, results show that chemical ecology is a valuable tool in better understanding host seeking in this tritrophic system. This new information on volatile production and dispersal by sawfly infested plants and host seeking behavior of these parasitoids establishes the basis for future exploration of optimal blends of behaviorally active compounds used by parasitoids in host seeking, which may allow for more effective control of the wheat stem sawfly.

CHAPTER 1

LITERATURE REVIEW

Introduction

Bracon cephi (Gahan) and *Bracon lissogaster* Muesebeck (Hymenoptera: Braconidae), are the two most effective biological control agents of the wheat stem sawfly (*Cephus cinctus* Norton), in the northern Great Plains of North America (Nelson and Farstad 1953).

These two parasitoids are native to North America (Ainslie 1920). A. B. Gahan (1918) initially described *B. cephi* as *Microbracon cephi*. This parasitoid was originally found attacking wheat stem sawfly larvae inside the stem of grasses (Criddle 1922). C. F. Muesebeck (1953) described *B. lissogaster* from grass stems infested with wheat stem sawfly from Montana. Even though both parasitoid species were originally reported only from sawfly infested grasses (Criddle 1922, Ainslie 1929, Muesebeck 1953); as new farming methods substituted the native wild grasses areas with wheat, the wheat stem sawfly was forced to change its original feeding habits from grasses to wheat (Farstad 1940, Davis 1955, Holmes 1978). These host specific parasitoids followed the wheat stem sawfly into wheat; however, this transition has not been as effective as that of their host (Criddle 1922, Davis et al. 1955). In 1948 *B. cephi* was reported in wheat in North Dakota (Munro et al. 1949) and in Canada (Nelson and Farstad 1953, Holmes et al. 1963). *B. lissogaster* was also reported by Somsen and Luginbill (1956) attacking wheat stem sawfly in wheat and barley (*Hordeum vulgare* L.).

Taxonomic classification of both parasitoid species:

Phylum: Arthropoda

Class: Hexapoda (Insecta)

Order: Hymenoptera

Suborder: Symphyta

Family: Braconidae

Subfamily: Braconinae

Tribe: Braconini

Genus: *Bracon*

Species: *B. cephi* (Gahan)

B. lissogaster Muesebeck

B. cephi and *B. lissogaster* are protelean host-specific parasitoids with two generations per year (bivoltine) (Nelson and Farstad 1953, Somsen and Luginbill 1956, Holmes et al. 1963). They are ectoparasitoids, living on the external surface (integument) of its host, and are considered idiobionts, immediately paralyzing their host and consuming it in the location and stage it is when attacked. (Nelson and Farstad 1953, Runyon et al. 2002, Weaver et al. 2004). Both of these parasitoid species have very similar biological characteristics (Runyon et al. 2001). Somsen and Luginbill (1956) discuss the specific characteristics of each stage of these parasitoids.

Biology of *Bracon cephi* and *Bracon lissogaster*

Egg

Eggs are approximately 0.86 mm in length, elongated in shape, and with a pale yellow coloration (Nelson and Farstad 1953). Adult females can lay one or several eggs per host on the larval integument or close to the larva. The developmental time for this stage is from one to two days.

Larva

The newly-hatched larvae are approximately 1mm in length. Their color is initially translucent, turning into a brownish coloration in later instars. Larvae go through five instars. They have no appendages, except for the antennae, and strongly sclerotized mouthparts (Nelson and Farstad 1953).

The newly emerged larva attaches to the integument of its host using its mandibles and immediately begins to feed. After completing feeding, the fully grown larva spins a light brown cocoon inside the wheat stem for overwintering. The time elapsed from the moment the larva hatches until it spins the cocoon is about 10 days.

Pupa

This parasitoid goes through a prepupal stage, which lasts about two days. The pupa is white in color and is found inside the cocoon. The pupal stage duration is about six days depending on temperature and soil moisture (Nelson and Farstad 1953).

Adult

Males are approximately 3.5 mm and females 4.1 mm in length. Their color is reddish testaceous, with antennae, eyes, ocelli, ovipositor, hind tibiae and tarsi black or blackish; wings sub-hyaline, stigma blackish and venation brownish (Gahan 1918).

Adults emerge after completing the pupal stage by chewing a hole through the wall of the cocoon. This occurs as early as in late June. Adults feed on droplet moisture from the leaves and on nectar (Nelson and Farstad 1953). Compared to their host they are strong flyers and can fly longer distances.

Parasitization is described by Nelson and Farstad (1953), Holmes et al. (1956), and Somsen and Luginbill (1956). Briefly, the parasitoid walks up and down the stem touching it with its antennae in search of the larva. After locating the host, the female inserts its ovipositor into the stem and paralyzes the sawfly larva depositing one to four eggs on the larva or close to it. After emerging from the egg, the larva will feed on the surface of the host and consume it. When the parasitoid larva matures, it spins a cylindrical cocoon that is attached lengthwise to the inside of the stem at each end by a disk-like plate. The parasitoid larvae pupate in the spring and the adults emerge by chewing a circular hole in the stem wall.

History and Distribution of the Parasitoid Host

The wheat stem sawfly, *C. cinctus* was first reported and described in 1872 by Edward Norton based on specimens from Colorado and using co-types from Nevada and California (Norton 1872). In 1890, Albert Koebele reared adults from larvae found in

grass stems in Almeida, California and that same year it was also found in Montana (Ainslie 1920). James Fletcher reported the first damage of this insect in wheat (*Triticum aestivum* L.) in 1895 from Souris, Manitoba and Moose Jaw, Saskatchewan (Riley and Marlatt 1891, Ainslie 1920).

This native North American insect [challenged by Ivie and Zinojev (1996) and Ivie (2001), who believe it to be introduced] today is known to be present throughout the northern Great Plains of North America, concentrating its heaviest damage in Alberta, Manitoba, Saskatchewan (Canada) and Montana, Wyoming, and North and South Dakota (USA) (Wallace and McNeal 1966, Weiss and Morrill 1992).

Taxonomic classification of the wheat stem sawfly:

Phylum: Arthropoda

Class: Hexapoda (Insecta)

Order: Hymenoptera

Suborder: Symphyta

Family: Cephidae

Subfamily: Cephinae

Tribe: Cephini

Genus: *Cephus*

Species: *C. cinctus* Norton

Biology of Wheat Stem Sawfly

Adult

This is a small sawfly (8-13 mm) with a narrow elongated and slightly compressed laterally black body with three characteristic longitudinal yellow bands across the abdomen. Wings are smoke colored and the legs are yellow. Females are noticeably larger than males and have a visible long, dark, saw-like ovipositor.

Adults of both sexes are reported to be weak fliers and rarely fly long distances at one time, but they often perform a peculiar hovering flight windward. When resting on a plant stem, they are normally found with their head downward and the legs aligned with the body (Ainslie 1920, Criddle 1922).

This species of sawfly is univoltine, having only one generation per year. Males are haploid having nine chromosomes and females diploid with 18 chromosomes. Unmated females will produce male progeny and mated females produce female progeny (arrhenotoky) (Holmes 1978).

The life span of an adult varies between 5 and 8 days, and these normally emerge from May to July. Adult males emerge before females (protandry), this ensures that most of the early females will be mated and that most of the eggs oviposited in the early part of the adult flight will be females, while most of the eggs deposited near the end of the flight period will be males. The emerging adults are sexually mature and ready for copulation and oviposition. Copulation is very brief, lasting less than a minute, during which the male holds to the back of the female with his abdomen curved down and under the female

ovipositor. The time passed from copulation to oviposition is usually only several hours for this species.

Oviposition

Weather conditions play a very important role in the process of oviposition. Light, temperature, humidity, wind, and host factors will determine when and where the sawflies will lay their eggs. The number of eggs produced by a female depends on the larval size, as an average a female produces approximately 33-50 eggs (Ainslie 1920, Holmes 1978).

Oviposition normally occurs during the day, with temperatures ranging from 20° to 36°C and under conditions of low wind. A female will reportedly lay only one egg per stem, however several other females can oviposit in the same stem (Nansen et al. 2005a, 2005b, Perez et al. 2006).

According to Holmes and Peterson (1960), one of the most important factors in the selection of the oviposition site by the sawfly is the stage of plant growth. They determined that females preferred the more tender part of the internode. Females, early in the oviposition period will select the elongating stems to infest. Later, as the stems reach their full length and maturity, they will select the less mature stems or tillers. Usually females oviposit in the second-last developing internode and as the stem grows, eggs are laid in progressively higher internodes. Another important factor is the diameter of the stem. Large stems are preferred over the more slender stems (Ainslie 1920, Morrill et al. 1992, 2000).

Ainslie (1920) and Wallace and McNeal (1966) both describe the oviposition process, as follows from Ainslie (1920): “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 a 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.”

Egg

The egg is normally oviposited freely inside the stem either in a stem cavity or in a hollow space produced by the ovipositor of the female. The newly laid egg is crescent shaped, with a milky white color, and fairly symmetrical. The size of the egg will depend upon the size of the female that produced the egg. Egg sizes range from 1 to 1.25 mm long and 0.33 to 0.42 mm wide (Ainslie 1920). The eggs will hatch in approximately 7 days and the larvae will escape its confinement in the shell using a series of convulsive movements.

Larva

Initially, larvae are transparent and colorless, until they begin feeding. After feeding commences they develop a green-yellow color. Larvae have a pale brown head, with dark brown four pointed mandibles, and have no legs. The caudal horn, which allows it to move within the stem, is brown and armed with a series of stout bristles (Wallace and McNeal 1966). Larval size varies from 8 to 14 mm in length and 1 to 2 mm in diameter.

After the larva emerges from the egg it immediately begins to feed on the parenchymous and vascular tissue inside the stem, forming a gallery above and below the place where the egg was deposited. Larvae generally move up the stem feeding on plant tissue and destroying other larvae and eggs that they might encounter, until only one individual will remain alive in each stem at plant maturity (Seamans et al. 1944).

Around late July or early August, most of the larvae which are in the upper internodes move down toward the base of the plant. The signal that triggers this downward movement is probably provided by the transmission of visible and infrared light through the stem wall of the plant at desiccation (Holmes 1978, 1982). When the larva reaches ground level it begins to prepare for diapause. First it cuts a V-shape groove (notch) around the inside of the stem above or below ground level (Runyon et al. 2002). The cut does not completely sever the stem, but it weakens and allows the wind to break it off when the plant is dry. Immediately under this cut, the larva forms a plug from frass and other plant material. The stem will usually break at the notch forming a cut stem that serves as an overwintering chamber. Inside this cut stem, the larva will spin a transparent hibernaculum where it will spend the winter (Ainslie 1920). The duration of

the larval stage can be as short as 30 days before entering diapause. Obligatory diapause is at least 90 days and the breaking of the diapause will depend on warming weather conditions. Larvae for this species have five instars (Farstad 1940, Holmes 1978). In late May of the following spring the larvae will molt into the pupal stage.

Pupa

The sawfly pupa is exarate, initially milk-white in color, slender and with an average length of 12 mm and a diameter of 1.5 mm. After the first day the legs and body turn darker until reaching a black color. The pupal stage lasts about 7 to 14 days and during the first couple of days the pupa is motionless after which it begins to move up and down the overwintering chamber (Ainslie 1920). After completing the pupal stage, the mature pupa changes into an adult, which will push its way through the frass plug, and emerge from the cut stem.

Host Plants

The wheat stem sawfly was probably originally a native grass feeder, and developed in large stem feral grasses, such as *Agropyron* species, rye grasses (*Lolium* spp.), timothy grass (*Phleum pratense* L.), and brome grass (*Bromus inermis* L.) (Ainslie 1920, Criddle 1922). In 1891, C. V. Riley and C. L. Marlatt stated, “The economic importance of this species arises from the fact that it may be expected at any time to abandon its natural food plants in favor of the small grains, on which it can doubtless successfully develop” (Ainslie 1920). A few years later, in 1895, these words came true when James Fletcher found *C. cinctus* damaging wheat in Saskatchewan and Manitoba (Ainslie 1920). From

that moment on, the damage to small grain crops, especially wheat continued to increase as large areas of native wild grasses were plowed and planted with wheat due to changes in farming methods (Farstad 1940, Davis 1955, Holmes 1978).

Taxonomic classification of the key wheat stem sawfly host plant:

Phylum: Streptophyta

Class: Liliopsida (Monocots)

Order: Poales

Suborder: Poinae

Family: Poaceae

Subfamily: Pooideae

Tribe: Triticeae

Genus: *Triticum*

Species: *T. aestivum* L.

With the passage of time *C. cinctus* has expanded its range of host plants feeding on nearly all the cultivated grains and many wild grasses. Oat (*Avena sativa* L.) appears to be resistant to the wheat stem sawfly, reportedly because of a deficiency in some essential nutrient needed by the sawfly (Farstad 1940).

Crop Damage

This insect has become a serious problem for wheat producers. The damage caused by the wheat stem sawfly in wheat has been consistently reported in the millions of

bushels and many millions of dollars are lost each year within the impacted areas of United States and Canada (Davis 1955, Weiss and Morrill 1992).

The yield losses produced by the sawfly are considered to be caused by both physiological and physical factors. A physiological factor such as the cutting of the vascular and parenchymous tissues of the plant by the tunneling action of the larvae reduces the transport of nutrients and water affecting the development of the kernels, the grain quality, and yield (Holmes 1954, 1977, Morrill and Kushnak 1996). The losses produced by this type of damage have been estimated in approximately 10-20% kernel weight reduction (Seamans et al. 1944, Munro et al. 1949, Holmes 1977). The cutting of an internal V-shaped notch or groove at the base of the plant by the larvae, weakening the stem and resulting in the lodging of the stem, constitutes a physical factor. This lodging makes the harvest of these stems difficult causing a reduction in the amount of grain that will be harvested, or contributes to an increase in harvesting costs because of the supplementary equipment required to recover the lodged stems (Seamans et al. 1944, Weiss and Morrill 1992, Morrill et al. 1992). The estimated losses from not being able to harvest the lodged heads are between 3-10% of the total grain (Seamans et al. 1944, Munro et al. 1949, McNeal et al. 1955).

Control Methods

The wheat stem sawfly, considered one of the most important insect pests in wheat and causing millions of dollars in losses every year, continues to affect the wheat production through the northern wheat producing regions of the United States and in the

Canadian prairies. The biology of the sawfly makes it a very difficult pest to control. Currently, there is no single effective method known to control this pest. Partial control has been obtained by combining different tactics such as solid stem varieties, delay of spring planting, swathing, tillage, and biological control. Since the initial detection of wheat stem sawfly damage, several control tactics have been investigated with variable results.

Chemical Control

Substantial research has been performed trying to determine the effectiveness of insecticidal control. Munro et al. (1949) tested five insecticidal dusts (Chlordane, DDT, Toxaphene, Parathion, BHC, and DDD) using a power duster. After two years of tests, they concluded that none of the chemicals produced satisfactory control of the sawfly. In 1962, Wallace indicated that he was able to obtain 73% control of the sawfly using granular heptachlor applied in the furrow with the wheat seed (Wallace 1962). Holmes and Peterson (1963) performed experiments trying to support these results, but concluded that there are important concerns related to the effectiveness of heptachlor in the control of the wheat stem sawfly. To be effective, heptachlor required light sawfly infestations restricted to the lower internodes of the stems. High levels of sawfly infestation and oviposition high in the stems (top two internodes) were considered to limit the control by the insecticide. Foliar insecticides Lorsban (chlorpyrifos), Furadan (carbofuran), and Warrior (lambda-cyhalothrin) were tested for sawfly control. Results determined no significant differences in their effect when compared to the controls (Blodgett et al. 1996). Other insecticides [Gaucho 75W (imidacloprid), Parathion 8E (ethyl parathion)]

have been evaluated for the control of *C. cinctus* and were found ineffective (Gall and Dogger 1967, Holmes 1978, Anonymous 1997).

Based on the biology of the wheat stem sawfly in which the larvae feed and spend their entire life inside the wheat stem, the use of insecticides to control this insect has been found to be ineffective (Munro et al. 1949, Holmes and Peterson 1963, Holmes 1978). The only life stage of this insect that lives outside the stem is the adult, which emerges over many weeks to mate and oviposit. Because of these limiting factors there are no registered insecticides for the control of this pest.

Cultural Control

Tillage: The soil around the infested wheat stubs provides an excellent insulation for the hibernating larva against extreme temperatures and also prevents loss of water. Tillage consists in exposing the sawfly cut stems on the surface of the soil to conditions causing the death of the larvae by freezing or desiccation (Callenbach and Hansmeier 1944). Both desiccation and freezing of the exposed stems have been considered important mortality factors for the sawfly larvae (Salt 1961). For this reason, this technique has showed potential as a pest management practice against the wheat stem sawfly (Holmes and Farstad 1956).

This practice has been tested for sawfly control in both winter and spring wheat using fall or spring tillage with different results. Sawfly larvae mortality of 90% has been reported from North Dakota using fall tillage (Weiss et al. 1987). Holmes and Farstad (1956) reported 99% larval mortality using fall tillage and 98 to 100% mortality with

spring tillage. However, factors such as proper timing, adequate surfacing of infested stubs, and reinstatement of diapause are very important for the effectiveness of this method (Salt 1946, Holmes and Farstad 1956, Morrill et al. 1993). McBride et al. (1989) reported 25% mortality and Holmes (1978) 35-70% mortality using spring tillage. Using fall tillage, Callenbach and Hansmeier (1944) reported 35-40% mortality.

Despite the variability in sawfly control obtained with this management practice, it is still implemented and considered by farmers an effective management tool (Morrill et al. 1993, Blodgett et al. 1996a).

However, there are negative effects caused by the improper use of this practice. Parasitoid populations tend to overwinter higher in the wheat stems rather than at the base of the stubble like the sawflies (Morrill et al. 1998, Runyon et al. 2002). The inverting of the surface soil produced by tilling buries most of the plant residue (especially the higher parts of the plant) under the soil from which parasitoids cannot emerge. Inappropriate tillage practices have been known to cause effects such as soil water loss, soil compaction, and erosion (Quisenberry et al. 2000, Cochran et al. 2006).

Plant Density and Spacing: Research by Luginbill (1958) demonstrated that wheat plots having wider row spacing (12 inches) suffer more cutting losses from sawflies than those with less spacing (3 or 6 inches), and higher wheat plant densities within the rows (42 and 21 g/row) have the lowest sawfly cuttings compared to lower density rows (10.5 and 5.25 g/row), independently of the row spacing. Kemp (1934) established an association between stem solidness and sawfly resistance in wheat. The more solid the stem, the higher its resistance to sawfly attack (Farstad 1940). Plant spacing and density

have an effect in the development of stem solidness. In plots where the rows were widely spaced stem solidness was greater and in plots with higher plant density within the rows, stem solidness was less (Luginbill 1958).

Crop Rotation: The practice of continuously planting wheat in the same area provides ideal conditions for the wheat stem sawfly (Callenbach and Hansmeier 1945, Butcher 1946). It is recommended to rotate wheat with immune or resistant crops such as oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), soybeans (*Glycine max* L.), sunflower (*Helianthus annuus* L.), and legumes or with other wheat stem sawfly resistant wheat varieties (McBride et al. 1996). The problem with this recommendation is that “economics associated with continuous spring wheat production often make crop rotation to a nonhost...unattractive to producers” (Weiss and Morrill 1992).

Trap Strips: This control method consists in planting around a wheat field a strip of variable width of a highly attractive crop or wheat variety to the wheat stem sawfly. The strip, with higher sawfly infestation, will be destroyed at the end of the sawfly flight period (Morrill et al. 2001a). These trap crops should have appealing characteristics to the sawflies in order to ensure effectiveness. Morphological plant characteristics such as large stem diameter and height are preferred by the sawflies (Morrill et al. 1992). Even though the system seems to work and there have been reports of the effectiveness of this technique (Morrill and Kushnak 1996, Morrill et al. 2001a), wheat growers are not very fond of destroying the grains from the trap strip (Goosey 1999).

Delayed Planting: Spring wheat planted late in the season avoids considerable damage because stem elongation begins after most adult sawflies have laid their eggs. On the other hand, fields that are planted early in the season receive the full impact of the sawfly infestation (Jacobson and Farstad 1952). However, the practice of planting late is known to make less efficient use of the critical soil moisture, resulting in lower crop yield (Morrill and Kushnak 1996, 1999). Fields with a history of heavy sawfly infestations are recommended to be planted last in the season (Morrill and Kushnak 1999).

Plant Resistance: The first person to relate sawfly resistance to the solidness of the stem was Christian Farstad (1940). He thought that the amount and consistency of the pith in the lumen of the wheat stem had a direct effect in the survivability of the larva. The first solid-stemmed wheat variety developed was “Rescue”, a hard red spring wheat created for resistance to the wheat stem sawfly (Platt et al. 1948). There were positive reports on the use of this variety against *C. cinctus*, showing a high degree of resistance (Holmes and Peterson 1962, Luginbill and Knipling 1969). It was later determined that the stem solidness of such wheat varieties depended on the weather conditions. Cloudy and wet weather condition resulted in the formation of more hollow stems and reduced resistance, conversely, clear and dry weather contributed to the formation of more solid stems with a higher resistance (Roemhild 1954). Over the years, new resistant varieties have been developed with improvements in stem solidness. Solid stem varieties such as Cutless, Glenman, Leader, Lew, Tioga, Fortuna, and Lancer have been created (McBride 1996), but there is varied acceptance of these solid stem varieties by the growers because of their reduced yield (Weiss and Morrill 1992). In other cases, semi-solid stem varieties

(Amidon, Rambo, Border, and Scholar) with a higher yield potential have also been developed (Blodgett et al. 1996).

Biological Control

This method of population suppression has been discussed for many years. The high variability in the level of mortality obtained (Somsen and Luginbill 1957, Smith 1959, Beirne 1972, Morrill et al. 1998, Runyon et al 2002) has created controversy about its efficacy. However, there have been reports of successful suppression by biological control agents (Streams and Coles 1965, Morrill et al. 1994, 1998).

For the wheat stem sawfly, three different types of organisms have been reported as possible biological control agents:

- ▶ Soil fungal pathogens in the genus *Fusarium* have been found to attack wheat stem sawfly larvae. Several species of pathogens (*F. graminearum*, *F. acuminatum*, *F. equiseti*, and *F. avenaceum*) have been determined to be highly lethal to sawfly larvae.

The problem with these pathogens is that they cause crown rot disease in wheat.

Possibilities of using these pathogens as biocontrol agents are further discussed in Wenda-Piesik et al. (2009).

- ▶ A predatory beetle, *Phyllobaenus dubius* (Wolcott) (Coleoptera: Cleridae) has been found emerging from wheat stubs infested with sawfly larva (Morrill et al. 2001b).

Larvae of this beetle have been found inside dissected wheat stubs. There is very little information about this beetle and more research is required to determine its biology and feeding behavior.

► Parasitic wasps are the most studied and probably the most effective biological control agents of the wheat stem sawfly. Several species of parasitoids have been confirmed to attack sawflies and limited material has been published about these parasitoids (Smith 1931, 1959, Streams and Coles 1965, Beirne 1972, Morrill et al. 1994, 1998), but there is still considerable research required to fully understand population dynamics.

Parasitoids of the Wheat Stem Sawfly: Originally, the wheat stem sawfly was distributed in feral grasses (Ainslie 1920, Criddle 1922) and the native parasitoids existed in a natural balance with this insect in non-cultivated habitats. When the native and wild grasses were destroyed by the new farming methods, the sawfly moved into wheat (Farstad 1940, Davis 1955, Holmes 1978), but the parasitoids were not able to rapidly adapt or synchronize their life cycle to the new conditions of the host plant, which altered the success of the parasitoids. Parasitoids had to initiate the process of adaptation to their host in a new environment (Morrill et al. 1998). With the passage of time a few parasitoids were able to adapt to the new conditions.

Several parasitoid species are reported in the United States from wheat stem sawfly species (Davis et al. 1955, Ivie, 2001). Some of these parasitoids are introduced species as part of a classical biological control program for this pest.

Collyria calcitrator Gravenhorst (Hymenoptera: Ichneumonidae) is an egg parasite of the European wheat stem sawfly, *Cephus pygmaeus* L. It is also known as a non-sustaining ectoparasite of *C. cinctus* larvae. This parasitoid was introduced from England where it was reported to control 75% of its native host. In 1930 it was released in Saskatchewan, Canada and between 1952 and 1954 it was released in Choteau, Montana

and in North Dakota, to control the wheat stem sawfly. However, none of these releases produced positive results. Very few parasitoids were recovered from the release sites initially and subsequent sampling recovered no individuals after two years (Smith 1931, 1961, Wallace and McNeal 1966). Nonetheless, this species has been reported to successfully control *C. pygmaeus* in Canada (Smith 1959).

Scambus detritus (Holmgren) (Hymenoptera: Ichneumonidae) was found on uncut wheat stems infested with *C. cinctus* (Holmes 1953). Low levels of parasitism have been reported from this parasitoid apparently due to overwintering in the upper portions of wheat stems where it is destroyed during harvest.

Pediobius nigratarsis (Thomson) (Hymenoptera: Eulophidae) is an internal parasitoid of *Cephus* larvae. It is present in the United States and widely distributed in Canada (Smith 1959). Even though levels of up to 45% of parasitism have been detected for this parasitoid, its efficiency seems to be affected by the presence of *C. calcitrator*, which apparently displaces it (Salt 1961, Smith 1959) or causes it to avoid ovipositing in *C. pygmaeus* hosts previously parasitized by *C. calcitrator* (Streams and Coles 1965).

There have been other introductions or redistributions of parasitoids to control sawflies, *Bracon terebrella* Wesm., *Pediobius uthaensis* (Crawford), *Heterosphilus cephi* Rohwer, but they all have failed to establish successfully (Davis et al. 1955, Smith 1961, Weiss and Morrill 1992).

From all these parasitoids, either native or introduced into the United States, *Bracon cephi* (Gahan) and *B. lissogaster* Muesebeck are considered to be the most effective and are commonly found parasitizing wheat stem sawflies in wheat fields.

These two parasitoids have been very successful in the utilization of the wheat stem sawfly in native grasses, but their effectiveness against the pest in wheat was initially very limited (Somsen and Luginbill 1956). There has been great variability in the reports of their effectiveness, ranging from 5 to 85% (Somsen and Luginbill 1956, Morrill et al. 1998, Runyon et al. 2002). Studies have been conducted to determine the causes for this variation in the effectiveness of these parasitoids. Holmes et al. (1963) found that parasitism by *B. cephi* depended on the synchronization between the sawfly and the parasitoid. High densities of sawfly larvae per stem have been found to be detrimental to the parasitoids. Under these conditions several female sawflies can lay an egg inside the stem of a single plant. Because the sawfly larvae present inside the stem will be bigger and better developed than the parasitoids, and because of their cannibalistic behavior, any of the unparasitized sawfly larvae may eat the parasitoid larva affecting the next parasitoid population (Weaver et al. 2005). Resistant wheat varieties are of varying suitability for parasitism because they reduce the survival of the sawfly larvae inside the stem, affecting the levels of parasitism (Holmes et al. 1963). Stem cutting by the sawfly larva, date of ripening of the host plant, soil moisture and temperature during the growing season, the development rate of the host plant, and tillage are some of the factors that have been found to have an impact on the levels of parasitism (Holmes et al. 1963, Runyon et al. 2002).

There is great need for additional research in this area that can provide valuable information on how to manipulate factors that are affecting the performance of these parasitoids. Recent publications have contributed important information on how to

reduce the effect of some of these factors and increase parasitism. Meers (2005) recommended leaving at least one-third of the standing crop residue to provide overwintering sites for the parasitoids, and harvesting only the wheat heads results in maximum parasitoid conservation. Runyon et al. (2002) determined that the elimination of intensive tillage in favor of light tillage or chemical fallow should result in greater parasitism. Morrill et al. (1998) found that parasitism levels were increased by releasing individuals from regions with high levels of parasitism into regions with low parasitism.

Plant Semiochemicals

Plants interact with other organisms through a complex system of chemical signals (semiochemicals). These chemical signals play a very important role in the survival of these plants, but perhaps one of the greatest mysteries related to the production of these plant volatiles is their true function. Our knowledge on the production and distribution of plant volatiles remains quite limited, although it has extended considerably over the past 15 years thanks to the invention and easier accessibility of valuable equipment (Heath and Manukian, 1992, Dudareva et al. 2004).

Each year there are considerable numbers of new compounds discovered from different plants, but there is little known about the function of most of these. For example, one of the most intensively studied plant compounds is isoprene; however, its function is still controversial (Sharkey et al. 2001). An important area of this complex semiochemical system is the release of volatiles from vegetative organs after they have been damaged by herbivores. Some of these volatile compounds are thought to be

specific for the plant species. Specific volatile compounds found in cabbage (Vuorinen et al. 2004), cucumber (Mercke et al. 2004), and maize (Degen et al. 2004) have been determined to work as indirect plant defenses specifically for each of these crops. These plant defenses are also responsible for attracting herbivores to the plants and also to attract biological control agents toward the herbivores, thus minimizing the damage to the plant (Paré and Tumlinson, 1999, Dicke and Van Loon, 2000). It is possible that herbivore-induced volatiles may also act as direct defenses, repelling (De Moraes et al. 2001, Kessler and Baldwin 2001) or intoxicating (Vancanneyt et al. 2001) herbivores and inhibiting pathogens.

Semiochemicals in Plant-Insect Systems

Within a plant-insect system, the response of the plant to insect herbivory by synthesizing and releasing volatile compounds provides important host location signals for the biological control agents to localize the herbivore (De Moraes et al. 2001). These chemical signals have other functions as well, they allow insects to detect their food, find mates, or locate a proper oviposition site. However, plants are not the only source of semiochemicals; insects are also known to produce signaling compounds (Dicke and van Loon 2000).

Plant semiochemicals typically produced when plants defend themselves from herbivorous arthropods (Tumlinson et al. 1992, Guerrieri et al. 1997, 2002) or from attacks by pathogens have been grouped in two categories, direct and indirect responses. Direct responses are those compounds that the plant produces which exert an effect on the herbivore or on the pathogen. Examples of some of these chemicals are toxic secondary

metabolites, such as nicotine or furanocoumarins (Reitz and Trumble 1996), oviposition deterrents, defensive proteins, and many other modes of action (Baldwin and Preston 1999, Hilker and Meiners 2002). Indirect responses are compounds that do not often have a direct effect on the pest; instead they attract parasitoids and predators (Turlings et al. 1990, Mayland et al. 2000, Colazza et al. 2004).

In the wheat system, there has been a considerable amount of work related to the production of volatile chemicals by wheat (Kemp and Andersen 1984, 1986, Gianoli and Niemeyer 1997, Quiroz et al. 1997, Fuentes-Contreras and Niemeyer 1998, Jiménez-Martínez et al. 2004, Peck 2004, Piesik et al. 2006, Lenardis et al. 2007), but comparatively little research conducted on induced compounds. In contrast, in bean plants (*Vicia faba* L. and *Phaseolus vulgaris* L.), the green stink bug, *Nezara viridula* L. has been found to induce the production of compounds which attract the scelionid parasitoid *Trissolcus basalis* (Wollaston) (Colazza et al 2004). The parasitoid *Pteromalus cerealellae* (Ashmead) uses chemical cues produced by its host the cowpea weevil, *Callosobruchus maculatus* F. when feeding (Mbata et al. 2004). The larval parasitoid *Hyssopus pallidus* (Askew) detects the volatiles emitted by apples (*Malus domestica* Borkhausen) when attacked by the codling moth, *Cydia pomonella* (L.) (Mattiacci et al. 1999). Guerrieri et al. (2002) found that volatile induction through herbivory on the roots of the broad bean plant (*Vicia faba* L.) played a key role in the flight orientation of the parasitoid *Aphidius ervi* Haliday. There are many examples of this volatile-driven behavior of parasitoids when detecting their hosts (Turlings et al. 1990, 1991, Vet and Dicke 1992, Cortesero et al. 1997, Conti et al. 2003).

Plants produce chemical volatiles, but these semiochemicals are also used by insects as pheromones, kairomones, or allomones. Pajares et al. (2004) analyzed the attractive response of the longhorn beetle *Monochamus galloprovincialis* Olivier to semiochemicals from the host and from the bark beetle *Ips* spp., determining that a blend of four semiochemicals from the bark beetle and the host compounds were highly attractive. Noldus et al. (1988) studied the role of sex pheromones of noctuid moths in the foraging behavior of parasitic *Trichogramma* spp while Gazit et al. (1996) determined the effect of a kairomone associated with the eggs of one host, *Spodoptera frugiperda* (J. E. Smith), showing that wasps were arrested by kairomones extracted from the bodies of the male and female moths.

Semiochemicals in the Wheat Stem Sawfly System

Research on the production of semiochemicals by wheat plants attacked by wheat stem sawfly and the effect of these volatiles on the sawflies is relatively recent. Based on the continuing need to find an effective method of control for this wheat pest, in recent years there has been investigation of chemical ecology for the wheat plant and this herbivore.

Cossé et al. (2002) studied the pheromone components of *C. cinctus* emanations using a gas chromatograph-electroantennogram detection system. They determined the presence of 13 active compounds that stimulated both male and female antennae. They also performed field bioassays in which they found attractiveness of both males and females to 9-acetyloxynonan.

Bartelt et al. (2002) investigated the cuticular lipids of the wheat stem sawfly determining that the most abundant components were n-alkenes and n-alkanes. This study was intended to provide information on the possible use of some of these compounds as pheromones, but the results were not very clear. The wheat stem sawfly appears to have very atypical pheromone biology and further research is needed. Other experiments determined that the major compounds of the male and female cuticular extracts produced no electrophysiological activity until degraded (Cossé et al. 2002).

Behavioral studies by Piesik et al. (2008) on the response of *C. cinctus* to specific wheat odors in a Y-tube olfactometer system has determined that females are highly attracted to (*Z*)-3-hexenyl acetate, (*E*)- β -ocimene, and (*Z*)-3-hexenol. Males were not attracted to any of these compounds. The results demonstrated that the responses by the female sawflies were concentration dependent.

Recent study (Peck 2004) of the differences in volatile production by sawfly infested and uninfested wheat plants has determined that sawfly damage induces quantitative changes in some of the volatiles produced by the wheat plants. No unique compounds were identified for infested plants. The compounds that were produced in different amounts between infested and uninfested plants were: 1-octen-3-ol, 2-undecanone, tridecane, 2-tridecanone, 2-pentadecanone, hexahydrofarnesyl acetone, benzyl alcohol, pentadecanal, hexahydropseudoionone, vanillin, 6-methyl-2-heptanone, geranyl acetone, 4-oxoisophorone, dihydroactinidiolide, 6-methyl-5-heptene-2-one, 1-tetradecene, 2,6-dimethyl-2,6-octadiene, phenylethanal, 1-hexanol, and an unknown compound (probably a furan based on mass spectral fragmentation).

Semiochemicals have been used effectively in developing control methods for important pest (Lewis et al. 1982, Turlings et al. 1995, Foster and Harris 1997, Cortesero et al. 2000, Khan et al. 2000). Within the wheat stem sawfly system there is urgent need for research focused on the development of effective control methods for this pest. As it has occurred with other pests, the use of chemical ecology within biological control might be a valuable tool in the process of achieving this goal.

Plants produce a considerable variety of volatile compounds (Hansson et al. 1999, Dudareva et al. 2004). Some specific volatile compounds that increase as a direct response to insect feeding play an important role in serving as attractants for parasitoids and predators (Pickett et al. 1997, Fatouros et al. 2008). There are numerous publications documenting this phenomenon of chemical cues used by biological control agents to locate their insect host. Based on the extensive information available about the use of volatile cues specifically by parasitoids to locate their hosts for many pest species (Drost et al. 1988, Herard et al. 1988a, 1988b, Eller et al. 1988a, 1988b, Turlings et al. 1989, Nodlus et al. 1990, Gohole et al. 2003, Pajares et al. 2004), we were interested in carefully analyzing this possibility with the wheat stem sawfly using two native braconid wasps (*B. cephi* and *B. lissogaster*).

In parasitoids, the survival of the species will depend on the ability of the ovipositing female to locate its host (Lewis et al. 1991). Most of the experimental data that we have been able to obtain on general parasitoid responses using plant volatiles is based solely on greenhouse and laboratory experiments. Lo Bue et al. (2004) studied the attraction of the parasitoid *Metaphycus* sp. nr. *flavus* (Howard) (Hymenoptera: Encyrtidae) to yucca

(*Yucca* sp.) leaves infested with the brown soft scale *Coccus hesperidum* L. (Hemiptera: Coccidae). They found that female parasitoids utilize volatile, water soluble compounds produced by the scale as chemical cues to locate suitable hosts. Mbata et al. (2004) investigated the role of semiochemicals in host location of the cowpea weevil *C. maculatus* (Coleoptera: Bruchidae) by the parasitoid *P. cerealellae* (Hymenoptera: Pteromalidae) determining that all the stimuli elicited by the beetles and infested seeds produced significantly better performance from the parasitoids.

Röse et al. (1998) explored the lack of specificity of systematically released compounds in response to different herbivore feeding on cotton plants (*Gossypium hirsutum* L.) and determined that the generalist parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) and the specialist parasitoid *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) were attracted to volatiles released by undamaged leaves on caterpillar damaged plants. There are many papers on the host recognition and learning ability of the parasitoid *M. croceipes* (Hymenoptera: Braconidae) using airborne semiochemical signals from its host plant (Eller et al. 1988a, b, 1992, Lewis et al. 1991, Röse et al. 1997).

However, very few papers have analyzed the effect of volatile chemicals under field conditions. If the idea is to determine the potential of this technique in applied entomology, it is necessary to expand these studies and explore applicability under field conditions. Many of the results obtained under laboratory conditions may have little relevance when the experiments are performed under the varying conditions that occur in the field (Hunter 2002).

De Moraes et al. (1998) studied the effect of plant emissions produced by herbivore damaged tobacco (*Nicotiana* sp.), cotton (*G. hirsutum* L.), and maize (*Zea mays* L.) plants on the response of the parasitoid *Cardiochiles nigriceps* Viereck under field conditions. Their results revealed that using the plant volatiles, the parasitoid is able to distinguish between two different herbivores, *Heliothis virescens* F. and *Helicoverpa zea* (Boddie). Thaler (1999) found that under field conditions, inducing plants with jasmonic acid increases parasitism of caterpillar pests and increases resistance against herbivorous insects. There are other studies on the effect of plant volatiles on parasitoids under field conditions, with differing outcomes (Kessler and Baldwin 2001, Sabelis et al. 2001, Hunter 2002, Carpenter and Jewett 2003, James 2003), but there is still much to be done to characterize these systems in the field.

This study explored sensory and behavioral responses of *B. cephi* and *B. lissogaster* to volatiles produced by wheat plants that may vary as a result of sawfly infestation. In addition, volatile production of active compounds was measured using an open volatile collection system in the greenhouse and in the field. The results provided new clues in understanding how larval parasitoids of stem borers (Morrill et al. 1994, Morrill et al. 1998, Runyon et al. 2002) cue on infested wheat stems despite the absence of unique signaling molecules. This lack of signaling molecules has been reported for other stem boring and gall forming herbivores in grasses (Ngi-Song and Overholt 1997, Turlings et al. 1998, Tooker and De Moraes 2007). This information provides insights into how to possibly enhance parasitoid efficacy in the future, as per Ockroy et al. (2001), Hern and

Dorn (2002), and James (2003), for the control of wheat stem sawfly in wheat fields by using behaviorally active compounds that are identified.

The results generated in this study contribute with valuable information to the foundations for understanding this complex wheat stem sawfly system and to the future development of an effective control method for this pest.

References

- Ainslie, C. N. 1920. The western grass-stem sawfly. U.S. Dept. Agric. Bull. 841.
- Anonymous. 1997. Wheat stem sawfly. Montana Crop Health Report. Special Edition. Montana Extension Service 10: 1-7.
- Baldwin, I. T. and C. A. Preston. 1999. The eco-physiological complexity of plant responses to insect herbivores. *Planta* 208: 137-145.
- Bartlett, R. J., A. A. Cossé, R. J. Petroski, and D. K. Weaver. 2002. Cuticular hydrocarbons and novel alkenediol diacetates from wheat stem sawfly (*Cephus cinctus*): natural oxidation to pheromone components. *J. Chem. Ecol.* 28:385-405.
- Beirne, B. P. 1972. The biological control attempt against the European wheat stem sawfly, *Cephus pygmaeus* (Hymenoptera: Cephidae), in Ontario. *Can. Entomol.* 104: 987-990.
- 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. *Mont. Crop Health Rep.* 9: 1-7.
- Butcher, F. G. 1946. The wheat stem sawfly. N. Dak. Agric. Col. Ext. Ser. Cir. A-94. 5 p.
- Callenbach, J. A. and M. P. Hansmeier. 1944. Wheat stem sawfly control in severely infested areas. Montana Extension Service in Agriculture and Home Economics, Circular No. 156.
- Callenbach, J. A. and M. P. Hansmeier. 1945. Wheat stem sawfly control. Montana Extension Service in Agriculture and Home Economics, Circular No. 164.
- Carpenter, J. E., and D. K. Jewett. 2003. Influence of herbivore-damaged corn and cotton in the field recruitment of braconids parasitoids from feral populations. *Fla. Entomol.* 86: 233-238.
- Church, N. S. 1955. Moisture and diapause in the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae). *Can. Entomol.* 87: 85-87.

- Cochran, V., J. Danielson, R. Kolberg, and P. Miller. 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.
- Colazza, S., A. Fucarino, E. Peri, G. Salerno, E. Conti, and F. Bin. 2004. Insect oviposition induces volatile emissions in herbaceous plants that attract egg parasitoids. *J. Exp. Biol.* 207: 47-53.
- 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. *Biol. 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. *J. Chem. Ecol.* 23: 1589-1606.
- Cortesero, A. M., J. O. Stapel, and W. J. Lewis. 2000. Understanding and manipulating plant attributes to enhance biological control. *Biol. Control* 17: 34-49.
- Cossé, A. A., R. J. Bartlett, D.K. Weaver, and B. W. Zilkowski. 2002. Pheromone components of the wheat stem sawfly: identification, electrophysiology and field bioassay. *J. Chem. Ecol.* 28: 407-423.
- Criddle, N. 1922. The western-stem sawfly and its control. Can. Dept. Agric. Pamphlet No. 6, Ottawa.
- Davis, E. G. 1955. Status of the wheat stem sawfly in the United States in 1954. N. D. Agric. Exp. Stn. 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. N. D. Agric. Exp. Stn. Bull. 18: 63-65.
- De Moraes, C. M., W. J. Lewis, P. W. Paré, H. T. Alborn, and J. H. Tumlinson. 1998. Herbivore-infested plants selectively attract parasitoids. *Nature* 393: 570-573.
- De Moraes, C. M., M. C. Mescheer, and J. H. Tumlinson. 2001. Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature* 410: 577-580.
- Degen, T., C. Dillmann, F. Marion-Poll, and T. C. J. Turlings. 2004. High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. *Plant Physiol.* 135: 1928-1938.

- Dicke, M., and J. J. A. van Loon. 2000. Multitrophic effects of herbivore induced plant volatiles in an evolutionary context. *Entomol. Exp. Appl.* 97: 237-249.
- 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 on host-searching behavior of *Microplitis croceipes* (Cresson), a larval parasitoid of *Heliothis*. *J. Chem. Ecol.* 14: 1607-1616.
- Dudareva, N., E. Pichersky, and J. Gershenzon. 2004. Biochemistry of plant volatiles. *Plant Physiol.* 135: 1893-1902.
- 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). *Environ. Entomol.* 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). *J. Chem. Ecol.* 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). *Physiol. Entomol.* 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.
- Fatouros, N. E, M. Dicke, R. Mumm, T. Meiners, and M. Hilker. 2008. Foraging behavior of egg parasitoids exploiting chemical information. *Behav. Ecol.* 19: 677-689.
- Foster, S. P. and M. O. Harris. 1997. Behavioral manipulation methods for insect pest-management. *Annu. Rev. Entomol.* 42: 123-146.
- Fritzsche, M. E. and C. J. Turlings. 2001. Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids. *Evol. Ecol. Res.* 3: 553-565.
- Fuentes-Contreras, E. and H. M. Niemeyer. 1998. Dimboa glucoside, a wheat chemical defense, affects host acceptance and suitability of *Sitobion avenae* to the cereal aphid parasitoid *Aphidius rhopalosiphi*. *J. Chem. Ecol.* 2: 371-381.

- Gahan, A. B. 1918. Description of a new hymenopterous parasite (Braconidae). Proc. Ent. Soc. Wash. 20: 18-19.
- Gall, A. and J. R. Dogger. 1967. Effect of 2,4-D on the wheat stem sawfly. J. Econ. Entomol. 60: 75-77.
- Gazit, Y., W. J. Lewis, and J. H. Tumlinson. 1996. Arrestment of *Telenomus remus* (Hymenoptera: Scelionidae) by a kairomone associated with eggs of its host, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Biol. Control 6: 283-290.
- Gianoli, E. and H. M. Niemeyer. 1997. Lack of costs of herbivory-induced defenses in a wild wheat: integration of physiological and ecological approaches. Oikos 80: 265-279.
- Gohole, L. S., W. A. Overholt, Z. R. Khan, L. E. M. Vet. 2003. Role of volatiles emitted by host and non-host plants in the foraging behaviour of *Dentichasmias busseolae*, a pupal parasitoid of the spotted stemborer *Chilo partellus*. Entomol. Exp. Appl. 107: 1-9.
- 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.
- Guerrieri, E., F. Pennacchio, and E. Tremblay. 1997. Effect of adult experience on in-flight orientation to plant and plant-host complex volatiles in *Aphidius ervi* Haliday (Hymenoptera: Braconidae). Biol. Control 10: 159-165.
- Guerrieri, E., G. M. Poppy, W. Powell, R. Rao, and F. Pennacchio. 2002. Plant-to-plant communication mediating in flight orientation of *Aphidius ervi*. J. Chem. Ecol. 28: 1703-1715.
- Heath, R. R. and A. Manukian. 1992. Development and evaluation of systems to collect volatile semiochemicals from insects and plant using a charcoal-infused medium for air purification. J. Chem. Ecol. 18: 1209-1226.
- Hamilton-Kemp, T. R. and R. A. Andersen. 1984. Volatile compounds from *Triticum aestivum*. Phytochemistry 23: 1176-1177.
- Hamilton-Kemp, T. R. and R. A. Andersen. 1986. Volatiles from winter wheat: identification of additional compounds and effect of tissue source. Phytochemistry 25: 241-243.

- Hansson, B., M. Larsson, and W. Leal. 1999. Green leaf volatile-detecting olfactory receptor neurones display very high sensitivity and specificity in a scarab beetle. *Physiol. Entomol.* 24: 121-126.
- 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. *J. Chem Ecol.* 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. *J. Chem Ecol.* 14: 1597-1605.
- Hern, A. and S. Dorn. 2002. Induction of volatile emissions from ripening apple fruits infested with *Cydia pomonella* and the attraction of adult females. *Entomol. Exp. Appl.* 102: 145-151.
- Hilker, M. and T. Meiners. 2002. Induction of plant responses to oviposition and feeding by herbivorous arthropods: a comparison. *Entomol. Exp. Appl.* 104: 181-192.
- Holmes, N. D. 1953. Note on *Scambus detritus* (Holmg) (Hymenoptera: Ichneumonidae), a new parasite of wheat stem sawfly, *Cephus cinctus* Nort. *Can. Entomol.* 85: 339.
- Holmes, N. D. 1954. Food relations of the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae). *Can. Entomol.* 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). *Can. J. Agric. Sci.* 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). *Can. J. Plant Sci.* 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. *Can. Entomol.* 94: 348-365.
- Holmes, N. D. and L. K. Peterson. 1963. Heptachlor as a systemic insecticide against the wheat stem sawfly, *Cephus cinctus* Nort. *Can. Entomol.* 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. *Can. Entomol.* 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. *Can. Entomol.* 109: 1591-1598.
- Holmes, N. D. 1978. The wheat stem sawfly. *Proc. 26th Annu. Mtg. Entomol. Soc. Alberta*, pp. 2-13.
- Holmes, N. D. 1982. Population dynamics of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), in wheat. *Can. Entomol.* 114: 775-788.
- Hunter, M. D. 2002. A breath of fresh air: beyond laboratory studies of plant volatile-natural enemy interactions. *Agric. For. Entomol.* 4: 81-86.
- Ivie, M. A. 2001. On the geographic origin of the wheat stem sawfly (Hymenoptera: Cephidae): a new hypothesis of introduction from northeastern Asia. *Am. Entomol.* 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. *Can. Entomol.* 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* Nort. (Hymenoptera: Cephidae). *Can. Entomol.* 84: 90-92.
- James, D. G. 2003. Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: methyl salicylate and the green lacewing, *Chrysopa nigricornis*. *J. Chem. Ecol.* 29: 1601-1609.
- Jiménez-Martínez, E., S. N. A. Bosque-Pérez, P. H. Berger, R. S. Zemetra, H. Ding, and S. D. Eigenbrode. 2004. Volatile cues influence the response of *Rhopalosiphum padi* (Homoptera: Aphididae) to barley yellow dwarf virus-infected transgenic and untransformed wheat. *Environ. Entomol.* 33: 1207-1216.
- Kemp, H. L. 1934. Studies on solid stem wheat varieties in relation to wheat stem sawfly control. *Sci. Agric.* 15: 30-38.
- Kessler, A., and T. Baldwin. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291: 2141-2144.

- 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 Manag. Sci.* 56:957-962.
- Lenardis, A. E., C. van Baren, P. Di Leo Lira, and C. M. Ghera. 2007. Plant-soil interactions in wheat and coriander crops driving arthropod assemblies through volatile compounds. *Europ. J. Agronomy* 26: 410-417.
- Lewis, W. J., D. A. Nordlund, R. C. Gueldner, P. E. Teal, and J. H. Tumlinson. 1982. Kairomones and their use for management of entomophagous insects. XIII. Kairomonal activity for *Trichogramma* spp. of abdominal tips, excretions, and a synthetic sex pheromone blend of *Heliothis zea* (Boddie) moths. *J. Chem. Ecol.* 8: 1323-1331.
- Lewis, W. J., J. H. Tumlinson, and S. Krasnoff. 1991. Chemically mediated associative learning: an important function in the foraging behavior of *Microplitis croceipes* (Cresson). *J. Chem. Ecol.* 17: 1309-1325.
- Lo Bue, P., L. D. Forster, J. G. Miller, S. Colazza, and R. F. Luck. 2004. Responses of *Metaphycus* sp. nr. *flavus* to semiochemicals released from a scale host, *Coccus hesperidum*. *Chemoecology* 14: 151-156.
- Luginbill, P. 1958. Influence of seeding density and row spacing on the resistance of spring wheat to the wheat stem sawfly. *J. Econ. Entomol.* 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.
- Mattiacci, L., E. Hutter, and S. Dorn. 1999. Host location of *Hyssopus pallidus*, a larval parasitoid of the Codling moth, *Cydia pomonella*. *Biol. Control* 15: 241-251.
- Mayland, H., D. C. Margolies, and R. E. Charlton. 2000. Local and distant prey related cues influence when an acarine predator leaves a prey patch. *Entomol. Exp. Appl.* 96: 245-252.
- Mbata, G. N., S. Shu, T. W. Phillips, and B. Ramaswamy. 2004. Semiochemical cues used by *Pteromalus cerealelle* (Hymenoptera: Pteromalidae) to locate its host, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Ann. Entomol. Soc. Am.* 97: 353-360.
- 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. *Agron. J.* 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.
- Mercke, P., I. F. Kappers, F. W. A. Verstappen, O. Vorst, M. Dicke, and H. J. Bouwmeester. 2004. Combined transcript and metabolite analysis reveals genes involved in spider mite induced volatile formation in cucumber plants. *Plant Physiol* 135: 2012-2024.
- Morrill, W. L., J. W. Gabor, and G. D. Kushnak. 1992. Wheat stem sawfly (Hymenoptera: Cephidae) damage and detection. *J. Econ. Entomol.* 85: 2413-2417.
- Morrill, W. L., J. W. Gabor, and D. Wichman. 1993. Mortality of the wheat stem sawfly (Hymenoptera: Cephidae) at low temperatures. *Environ. Entomol.* 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. *J. Econ. Entomol.* 87: 1373-1376.
- Morrill, W. L. and G. D. Kushnak. 1996. Wheat stem sawfly (Hymenoptera: Cephidae) adaptation to winter wheat. *Environ. Entomol.* 25: 1128-1132.
- Morrill, W. L., G. D. Kushnak, and J. W. Gabor. 1998. Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) in Montana. *Biol. 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. *J. Agric. Urban Entomol.* 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). *Environ. Entomol.* 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). *J. Entomol. Sci.* 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). *J. Kansas Entomol. Soc.* 74: 181-183.

- Munro, J. A., W. Nostdahl, and R. L. Post. 1949. Wheat stem sawfly. North Dakota Agr. Col. Bimon. Bull. 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. Can. Entomol. 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. Can. Entomol. 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. Can. Entomol. 85: 103-107.
- Ngi-Song, A. J., and W. A. Overholt. 1997. Host location and acceptance by *Cotesia flavipes* Cameron and *C. sesamiae* (Cameron) (Hymenoptera: Braconidae), parasitoids of African gramineous stemborers: role of frass and other host cues. Biol. Control 9: 136-142.
- Noldus, L. P. J. J. 1988. Response of the egg parasitoid *Trichogramma pretiosum* to the sex pheromone of its host *Heliothis zea*. Entomol. Exp. Appl. 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. J. Chem. Ecol. 16: 3531-3544.
- Norton, E. 1872. Notes on North American Tenthredinidae with descriptions of new species. Trans. Am. Entomol. Soc. 4: 77-86.
- Ockroy, M. L. B., T. C. J. Turlings, P. J. Edwards, M. E. F. Hoballah, L. Ambrosetti, P. Bassetti, and S. Dorn. 2001. Response of natural populations of predators and parasitoids to artificially induced volatile emissions in maize plants (*Zea mays* L.). Agri. Forest Entomol. 3: 201-209.
- Pajares, J. A., F. Ibeas, J. J. Diaz, and D. Gallego. 2004. Attractive responses by *Monochamus galloprovincialis* (Col., Cerambycidae) to host and bark beetle semiochemicals. Blackwell Verlag, Berlin 128: 633-638.
- Paré, P. W. and J. H. Tumlinson. 1999. Plant volatiles as a defense against insect herbivores. Plant Physiol. 121: 325-331.

- Peck, G. E. 2004. Potential semiochemicals of wheat (*Triticum aestivum* L.) induced by oviposition and feeding of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae) [Master's Thesis]. Montana State University, Bozeman, Montana. 65 p.
- Perez, J., D. K. Weaver, and W. L. Morrill. 2006. Infestation of wheat and downy brome grass by wheat stem sawfly and subsequent larval performance. *Environ. Entomol.* 35: 1279-1285.
- Pickett, J. A., L. J. Wadhams, and C. M. Woodcock. 1997. Developing sustainable pest control from chemical ecology. *Agric. Ecosys. Environ.* 64: 149-156.
- Piesik, D., D. K. Weaver, G. E. Peck, W. L. Morrill. 2006. Mechanically-injured wheat plants release greater amounts of the secondary metabolites linalool and linalool oxide. *J. Plant Prot. Res.* 46: 29-39.
- Piesik, D., D. K. Weaver, J. B. Runyon, M. Buteler, G. E. Peck, and W. L. Morrill. 2008. Behavioural responses of wheat stem sawflies to wheat volatiles. *Agric. For. Entomol.* 10: 245-253.
- Platt, A. W., C. W. Farstad, and J. A. Callenbach. 1948. The reaction of Rescue wheat to sawfly damage. *Sci. Agric.* 28: 154-161.
- Quiroz, A., J. Pettersson, J. A. Pickett, L. J. Wadhams, H. M. Niemeyer. 1997. Semiochemicals mediating spacing behavior of bird cherry-oat aphid, *Rhopalosiphum padi*, feeding on cereals. *J. Chem Ecol* 23: 2599-2607.
- 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. *J. Entomol. Sci.* 35: 327-333.
- Reitz, S. R., and J. T. Trumble. 1997. Effects of linear furanocoumarins on the herbivore *Spodoptera exigua* and the parasitoid *Archytas marmoratus*: host quality and parasitoid success. *Entomol. Exp. Appl.* 84: 9-16.
- 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.

- Röse, U. S. R., H. T. Alborn, G. Makranczy, W. J. Lewis, and J. H. Tumlinson. 1997. Host recognition by the specialist endoparasitoid *Microplitis croceipes* (Hymenoptera: Braconidae): role of host- and plant-related volatiles. *J. Insect Behav.* 10: 313-329.
- Röse, U. S. R., W. J. Lewis, and J. H. Tumlinson. 1998. Specificity of systematically released cotton volatiles as attractants for specialist and generalist parasitic wasps. *J. Chem. Ecol.* 24: 303-319.
- 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 in Montana. *J. Econ. Entomol.* 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.
- Salt, R. W. 1946. Moisture relationships of the wheat stem sawfly (*Cephus cinctus* Nort.) I. Some effects of desiccation. *Sci. Agric.* 26: 622-630.
- Salt, R. W. 1961. Principles of insect cold-hardiness. *Annu. Rev. Entomol.* 6: 55-74.
- 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 Entomol. Soc. Annu. Rpt.* 75: 10-15.
- Sharkey, T. D., X. Y. Chen, and S. Yen. 2001. Isoprene increases thermotolerance of fosmidomycin-fed leaves. *Plant Physiol.* 125: 2001-2006.
- Smith, C. W. 1931. Colonization in Canada of *Collyria calcitrator* (Hym.: Ichn.), a parasite of the wheat stem sawfly. *Bull. Entomol. Res.* 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). *Can. Entomol.* 91: 697-700.
- Smith, R. W. 1961. Notes on parasites of the wheat stem sawfly, *Cephus pygmaeus* (L.) (Hymenoptera: Cephidae) from continental Europe. *Can. Entomol.* 93: 714-717.
- Somsen, H. W. and P. Luginbill. 1957. *Bracon lissogaster* Mues., a parasite of the wheat stem sawfly. *USDA Tech. Bull.* No. 1153.

- Streams, F. A. and L. W. Coles. 1965. Wheat stem sawfly parasites, *Collyria calcitrator* and *Pediobius nigratarsis* in Eastern United States. J. Econ. Entomol. 58: 303-306.
- Thaler, J. S. 1999. Jasmonate-inducible plant defenses cause increased parasitism of herbivores. Nature 399: 686-688.
- Tooker, J. F. and C. M. De Moraes. 2007. Feeding by Hessian fly [*Mayetiola destructor* (Say)] larvae does not induce plant indirect defenses. Ecol. Entomol. 32: 153-161.
- Tumlinson, J. H., T. C. Turlings, and W. J. Lewis. 1992. The semiochemical complexes that mediate insect parasitoid foraging. Agric. Zool. Rev. 5: 221-250.
- Turlings, T. C., J. H. Tumlinson, and W. J. Lewis. 1990. Exploitation of herbivore induced plant odors by host-seeking parasitic wasps. Science 250: 1251-1253.
- Turlings, T. C., J. H. Tumlinson. 1991. Do parasitoids use herbivore-induced plant chemical defenses to locate hosts? Fla. Entomol. 74: 42-50.
- Turlings, T. C., J. H. Loughrin, P. J. McCall, U. S. R. Röse, W. J. Lewis, and J. H. Tumlinson. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. Proc. Natl. Acad. Sci. USA. 92: 4169-4174.
- Turlings, T. C. J., M. Bernasconi, R. Bertossa, F. Bigler, G. Caloz, and S. Dorn. 1998. The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. Biol. Control 11:122-129.
- Vancanneyt G., C. Sanz, T. Farmaki, M. Paneque, F. Ortego, P. Castanera, and J. J. Sanchez-Serrano. 2001. Hydroperoxide lyase depletion in transgenic potato plants leads to an increase in aphid performance. Proc. Natl. Acad. Sci. USA 98: 8139-8144.
- Vet, L. E. and M. Dickie. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. Annu. Rev. Entomol. 37: 141-172.
- Vuorinen T., A. M. Nerg, M. A. Ibrahim, G. V. P. Reddy, and J. K. Holopainen. 2004. Emission of *Plutella xylostella*-induced compounds from cabbages grown at elevated CO₂ and orientation behavior of the natural enemies. Plant Physiol. 135: 1984-1992.
- Wallace, L. E. 1962. Field-plot tests of chemicals for wheat stem sawfly control. J. Econ. Entomol. 55: 908-912.

- Wallace, L. E., and F. H. McNeal. 1966. Stem sawflies of economic importance in grain crops in the United States. USDA Tech. Bull. 1350: 1-50.
- 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. Biol. Control 34: 1-11.
- Weiss, M. J. and W. L. Morrill. 1992. Wheat stem sawfly (Hymenoptera: Cephidae) revisited. Am. Entomol. 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. Environ. Entomol. 38: 387-394.

CHAPTER 2

ELECTROANTENNOGRAM RESPONSES OF PARASITOIDS *BRACON CEPHI*
AND *BRACON LISSOGASTER* TO SYNTHETIC WHEAT VOLATILES
MATCHING THOSE RELEASED BY WHEAT PLANTS INFESTED
WITH WHEAT STEM SAWFLY

Introduction

Bracon cephi (Gahan) and *B. lissogaster* Muesebeck (Hymenoptera: Braconidae), are two highly effective parasitoids of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), one of the most destructive insect pests of wheat (*Triticum aestivum* L.) in the northern Great Plains of North America (Weiss and Morrill 1992, Morrill and Kushnak 1996, Buteler et al. 2008). This pest has been reported to cause losses in the wheat production of this area in the order of tens of millions of dollars each year (Weiss and Morrill 1992, Weaver et al. 2004, 2005). The biology of this insect, a stem borer with all immature stages inside the wheat stem, makes it very difficult to control using conventional methods. This has created a serious and continuing problem for the wheat producers. There is great need to develop new effective methods to control this pest and to improve the existing ones.

It is well known that insects use chemical signals from the environment to find food, adequate oviposition sites, mates, or hibernation sites (Janssen et al. 1997, Landolt and Phillips 1997, Agelopoulos et al. 1999, Ode 2006, Randlkofer et al. 2007). Chemical ecology is an effective tool to understand the behavior of insect pests (Byers 1989, Pickett

et al. 1992), and it has been exploited in different crops for insect pest control (Lewis et al. 1982, Turlings et al. 1995, Foster and Harris 1997, Cortesero et al. 2000, Khan et al. 2000). There is abundant information supporting the capability of plants to produce specific volatile compounds or to modify the amounts of volatile compounds produced when attacked by herbivorous pests (Du et al. 1998, Dicke and van Loon 2000, Kessler and Baldwin 2001, James 2003, Dudareva et al. 2004, Degenhardt 2009). In recent years, there has been considerable documentation of the importance of these induced plant chemicals in the ability of parasitic Hymenoptera to find their hosts (Eller et al. 1988, Turlings et al. 1990, Tumlinson et al. 1993, Lewis et al. 1994, De Moraes et al. 1999, Turlings et al. 1998, Tentelier and Fauvergue 2007). The manipulation of compounds produced by herbivore-attacked plants or of pheromones produced by host insects may offer methods to enhance the effectiveness of biological control agents in some crops (Pickett et al. 1997, Agelopoulos et al. 1999, Rott et al. 2005, Ode 2006).

One of the exploratory techniques used in chemical ecology is the identification of sensory responses of insects to specific plant volatile compounds using electrophysiology. The electroantennogram (EAG) system is one method that is used for this purpose. This technique developed by Schneider (1957) on the antenna of male silk moths (*Bombyx mori* L.), consists of recording the sums of the electric potentials produced by the receptor neurons of an insect antenna as it is stimulated by a compound (Eltz and Lunau 2005). The system has been widely used in different crops [broad bean, *Vicia faba* L. (Du et al. 1998), cabbage, *Brassica oleracea* L. (Coleman et al. 1997, Rojas 1999), corn, *Zea mays* L. (Hibbard et al. 1997, Gouinguené et al. 2005), cotton, *Gossypium hirsutum* L. (Dickens

1984, Gouinguené et al. 2005), cowpea, *Vigna unguiculata* L. (Gouinguené et al. 2005), oak, *Quercus* spp. (Kozłowski & Visser 1981), Chinese tea (Baoyu and Zongmao 2002, Baoyu and Baohong 2007), and a large complex of other plants (Visser 1979, Jyothi et al. 2002) to assess the antennal response of insects to volatile compounds produced by the plants. This method constitutes an effective and convenient way to assess the overall sensitivity of insects to a wide variety of compounds at varying concentrations (Thiery and Marion-Poll 1998). Electroantennogram studies with different parasitoid species have determined that variations in the concentration of the volatile compounds tested can generate different responses from the antenna of the parasitoids (Salom et al. 1991).

Hamilton-Kemp and Andersen (1984), Buttery et al. (1985) and Piesik et al. (2008), have characterized the production of specific volatile compounds by wheat plants. Piesik et al. (2008) determined that four of the seven reliably detected wheat compounds [(*Z*)-3-hexenyl acetate, (*Z*)-3-hexanol, 6-methyl-5-heptene-2-one, and (*E*)- β -ocimene] have been found to cause behavioral responses in female wheat stem sawflies. In closed volatile collections in the greenhouse, differences in the amounts of these compounds produced by sawfly infested and uninfested ‘McNeal’ wheat plants have been detected (Peck 2004).

Because of our interest in understanding and trying to improve the activity of *B. cephi* and *B. lissogaster* in the wheat stem sawfly system, electrophysiological responses of the antenna of these parasitoids to synthetic compounds matching those produced by wheat were analyzed. Some of the compounds identified by Peck (2004) and Piesik (2008) using closed volatile collection systems, as well as several reported in Chapter 4 using volatile collections from an open system, were examined at three different

concentrations using an electroantennogram system for sensory activity in these parasitoids.

Objectives

1. Determine if compounds produced by wheat plants infested by wheat stem sawfly generate electrophysiological responses from the antenna of parasitoids *B. cephi* and *B. lissogaster*.
2. Establish if there is a consistent pattern of responses from the parasitoid antennae to a standard series of concentrations of these tested compounds.

Test Hypotheses

Test Hypothesis 1: Compounds produced by sawfly infested wheat plants generate electrophysiological responses from the antenna of *B. cephi* and *B. lissogaster*.

Test Hypothesis 2: There is a consistent pattern in responses from parasitoid antenna to a series of concentrations of specific tested compounds.

Materials and Methods

Insects

The parasitoids used in the experiment were collected during the fall from postharvest wheat stubble in various fields throughout Montana from 2004 through 2007. The collected wheat stubble was used to obtain parasitoids via two different methods.

Method 1: Collected wheat stubble was separated into individual stems and each one was split open along the entire length of the stem. Sections of the stems with parasitoid cocoons were placed in 8.5 cm diameter plastic Petri dishes (Fisher Scientific, Pittsburgh, Pennsylvania) and stored in a refrigerator at 4°C until mid or late spring of the following year. This interval in cold storage allowed the parasitoids to complete their obligatory diapause. After completion of diapause, groups of approximately one hundred parasitoid cocoons were moved out of the refrigerator into room temperature and placed inside glass jars with a fine metal mesh lid. The cocoons were misted with tap water twice a week to maintain humidity. The number of parasitoid cocoons moved out of the refrigerator to obtain experimental adult parasitoids for the behavioral studies depended on the number available, on the percentage of emergence of the previous group, and on the timing of the emergence of the adult parasitoids. Limitations in the number of parasitoids available in some opportunities required the use of all the cocoons present in storage at a time. If the number of parasitoid cocoons available in storage were high, some cocoons could remain stored until the following experiment. If the percentage of emergence from a specific group of parasitoid cocoons was low, it was necessary to increase the number of cocoons moved out of the refrigerator in order to acquire the number of adults needed.

Method 2: Wheat stubble collected in bulk during the fall from the same locations was placed in 170 liter plastic barrel liners and stored in a cold storage room at 4°C until mid or late spring of the following year. After storage exceeded the duration of obligatory diapause, the plastic barrel liner was moved out of cold storage to room temperature.

Each barrel liner moved out of cold storage was prepared for parasitoid emergence as described by Meers (2005).

All parasitoids that emerged from these methods were collected and placed inside 1 liter glass Mason® jars with filter paper lining the bottom and a fine metal mesh lid with filter paper under the mesh for containment and ventilation. A glass vial containing sugar dissolved in distilled water (0.25 g ml^{-1}) was sealed with a cotton plug. This moist plug served as a food source for the adult parasitoids.

Electroantennogram Experiments

Adult female or male parasitoids obtained from the previously described methods were used in the experiment. Parasitoid preparation consisted in excising the head from the body and arbitrarily removing one of the antennae from the head. Using fine microdissection scissors, one third of the head (the side with the removed antenna) was longitudinally excised to improve the electrical conductance. This parasitoid head preparation was placed on one of the metal gold electrodes (conducting electrode) and the tip of the antenna on the other electrode (recording electrode). An electrically conductive gel (Spectra 360, Parker Laboratories Inc., Fairfield, New Jersey) was used on each electrode to improve the electrical connection. The two gold electrodes were connected to a micromanipulator, 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 (Bjostad 1998). The amplifier then transferred the information to a computer interface with an EAG version 2.7 software (Syntech NL 2001, Hilversum,

The Netherlands). This is a consistently used apparatus that represents the existing standard for insect electrophysiological research (Birkett et al. 2004)

A constant stream of purified, humidified air (20 ml sec^{-1}) was delivered onto the antenna. The test compound was delivered onto the antenna in the form of an airborne stimulus released from filter paper treated with $10 \mu\text{l}$ of the test compound and placed inside a glass pipette connected to a controlled air flow (Birkett et al. 2004). The compound was delivered in a specific 0.4 second puff of air, mixed into the constant stream of air. The puff duration was sufficient to stimulate the antenna to its maximum voltage deflection. The electrical response of the antenna to the test compound was detected by the recording electrode and transferred to the amplifier, which increased the signal and delivered the information to the computer interface where it was recorded by the software program (Syntech 1998, Bruce and Cork 2001).

For this experiment, ten synthetic compounds were used: (*Z*)-3-hexenyl acetate (Bedoukian Research Inc., Danbury, CT), hexahydrofarnesyl acetone (Bedoukian Research Inc., Danbury, CT), 6-methyl-5-heptene-2-one (99%, Aldrich Chem. Co., Milwaukee, Wisconsin), (*Z*)-3-hexenol (98%, Aldrich Chem. Co., Milwaukee, Wisconsin), α -pinene (98%, Aldrich Chem. Co., Milwaukee, Wisconsin), benzaldehyde (98%, Aldrich Chem. Co., Milwaukee, Wisconsin), (*E*)-2-hexenal (99%, Aldrich Chem. Co., Milwaukee, Wisconsin), (*E*)-2-hexenyl acetate (98%, Aldrich Chem. Co., Milwaukee, Wisconsin), (*E*)- β -ocimene (70%, Fluka Chemika, Aldrich Chem. Co., Milwaukee, Wisconsin), and decanal (98%, Aldrich Chem. Co., Milwaukee, Wisconsin). These compounds, known to be produced by wheat plants (Hamilton-Kemp and Andersen

1984, Buttery et al. 1985), were tested on the antennae of male and female *B. cephi* and *B. lissogaster* parasitoids. Only one puff of one specific concentration of the tested compound was delivered onto the antenna at a time. Compounds were tested on the parasitoid antennae randomly. Three specific concentrations of each compound were tested (0.01 ng, 100 ng, and 10000 ng). The concentrations were always tested in increasing amounts starting with the lowest. A variable number of males and females of each species (based on availability) were tested for each compound and concentration. The specific number of males and females tested for each compound and each concentration is presented in Appendix A and in all figures. The excised parasitoid head with the antenna survived for approximately 1 hr. Puffs of hexane (Certified A.C.S. Spectranalyzed[®], Fisher Scientific, New Jersey) before and after each stimulus delivery were used as stabilizing controls. Hexane is not produced by wheat plants and it has a low stimulatory effect on the parasitoid antenna. The typical depolarization of the antennal receptors by the hexane puff ranged from 0.2 through 0.8 millivolts.

Due to the inconsistent nature of the parasitoid emergence and the short-lived antennae, the data was obtained sporadically over a long period of time.

Statistical Analysis

The electroantennogram data obtained from the EAG software was transformed into response ratios using the control responses as a base line. The data was transformed using a log₁₀ transformation and analyzed using a t-test. A simple linear regression analysis was performed to determine the relationship between antennal response and the concentration of each compound tested. Comparisons between male and female

electrophysiological responses to each tested compound were analyzed using a t-test. All statistical analyses were performed in SAS (SAS 9.2, 2007). All the data presented in Figures and Tables are the original data that has not been transformed.

Results

The electrophysiological responses obtained from the experimental trials determined that there are concentrations of the tested wheat compounds that generated significant responses from the antennae of *B. cephi* and *B. lissogaster* parasitoids. Antennal responses from male *B. cephi* parasitoids to all the compounds except (Z)-3-hexanol, 6-methyl-5-heptene-2-one and decanal were not statistically analyzed because of the low number of individuals that were available for testing (Appendix E). The inavailability of sufficient number of parasitoids did not allow for reliable statistical analyses. We chose to not conduct any comparisons for datasets with less than 3 tested individuals. It was only possible to statistically analyze the responses from female *B. cephi*, male *B. lissogaster*, female *B. lissogaster*, and only the three previously mentioned compounds for male *B. cephi* (Appendices B, C, D, and E).

The profiles obtained for each of the tested compounds determined that antennae of female *B. cephi* significantly responded to six compounds, antennae from female *B. lissogaster* significantly responded to four, and antennae from male *B. lissogaster* significantly responded to five of the tested compounds.

(Z)-3-hexenyl acetate

The antennae of female *B. cephi* parasitoids exhibited a significant response ($P = 0.004$, < 0.0001 , and < 0.0001) to all three of the tested concentrations (0.01, 100, and 10000 ng respectively) of this compound (Appendix B). The strongest response ratio (3.7) was produced in response to the greatest concentration (10000 ng) of (Z)-3-hexenyl acetate. There is a positive relationship ($F = 11.35$, $P = 0.001$) between the tested compound concentrations and the antennal response of *B. cephi* females (Appendix B). The response ratios increased proportionally as the concentration of (Z)-3-hexenyl acetate increased (Appendix B).

Female *B. lissogaster* parasitoids exhibited a similar response. The antennal responses of the females of this parasitoid species to all three of the tested compound concentrations were significantly ($P = 0.02$, 0.002 , and 0.0002) greater than to the controls (Appendix C). The strongest antennal response ratio (3.8) was to the greatest compound concentration (Figure 2.1). There is a positive relationship between the compound concentrations and the antennal response of *B. lissogaster* females ($F = 5.10$, $P = 0.03$). An increase in the concentration of the tested compound generated a stronger response from the parasitoid antennae (Figure 2.1).

Male *B. lissogaster* antennae showed a significantly ($P = 0.03$ and 0.004) higher response only to the greater concentrations (100 and 10000 ng) of (Z)-3-hexenyl acetate (Appendix D). The strongest response ratio (1.6) was generated by the highest compound concentration (Figure 2.1). There is a positive relationship between

concentration and antennal response ($F = 7.96$, $P = 0.008$), with the response ratio increasing as the concentration of the compound increases.

Female *B. cephi* antennal response (2.76 mV) to (*Z*)-3-hexenyl acetate was found to be significantly stronger than males (1.27 mV) ($t = 5.48$, $P < 0.0001$). Female *B. lissogaster* antennal response (3.18 mV) was also found to be stronger than males (1.22 mV) ($t = 4.48$, $P = 0.0001$) (Table 2.1 and 2.2).

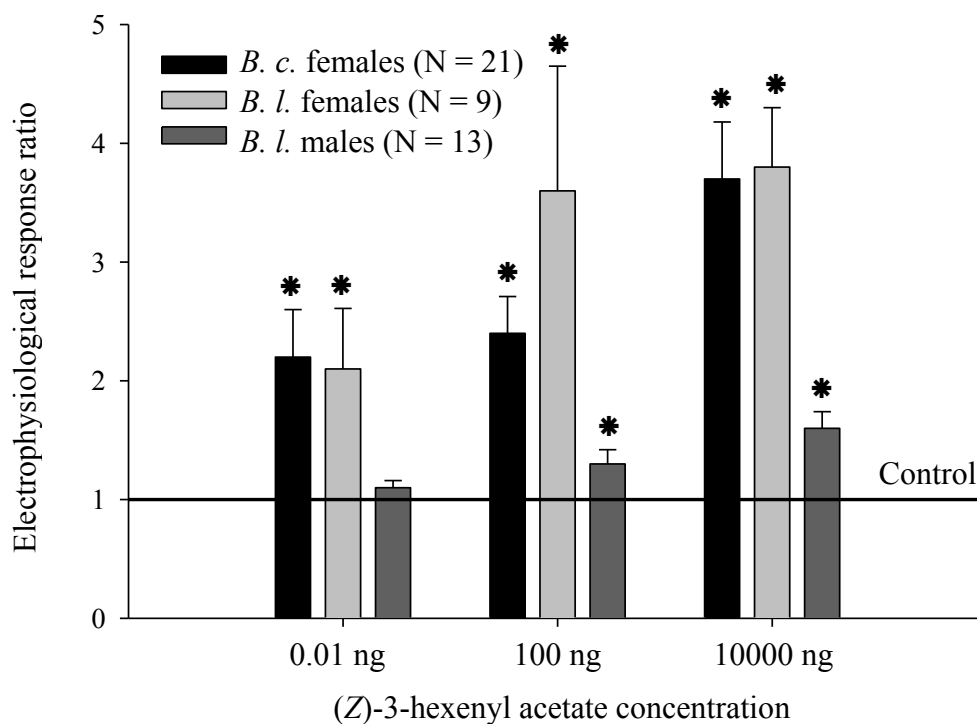


Figure 2.1. Electrophysiological response ratios from the antennae of female *B. cephi* and *B. lissogaster* and male *B. lissogaster* parasitoids to three concentrations of (*Z*)-3-hexenyl acetate. *: Statistically significant ($\alpha = 0.05$).

There were not enough male *B. cephi* parasitoids to perform a reliable statistical analysis of their response to this compound (Appendix E).

Hexahydrofarnesyl acetone

This compound, unlike the others, generated an unexpected response from the antennae of both parasitoid species. There were stronger electrophysiological responses to lower concentrations of the compound and weaker responses to the higher concentrations (Figure 2.2).

There was a significant response from *B. cephi* female antennae to all three of the tested concentrations (0.01, 100, 10000 ng) of this compound ($P < 0.0001$, 0.008, and 0.02, respectively) (Appendix B). In contrast with the other tested compounds, the strongest response ratio (1.8) was generated by the lowest concentration (0.01 ng) of hexahydrofarnesyl acetone and the weakest response (0.8) from the highest concentration (10000 ng) (Figure 2.2). There was a significant negative relationship between the compound concentration and the antennal response from the *B. cephi* females ($F = 36.39$, $P < 0.0001$) (Appendix B). This response is inversely proportional, as the concentration of hexahydrofarnesyl acetone increases the response ratio is weaker (Figure 2.2).

The *B. lissogaster* female parasitoids exhibited a similar pattern of response as the *B. cephi* females. There is a negative relationship between compound concentration and antennal response for these female parasitoids ($F = 5.81$, $P = 0.03$) (Appendix C). Only the antennal response to the lowest compound concentration was significantly greater than the control for these parasitoids ($P = 0.02$) (Figure 2.2). The strongest response ratio (2.0) was produced by the lowest compound concentration.

B. lissogaster males had the same response pattern as both *B. cephi* and *B. lissogaster* females, a significantly higher response to the lower concentrations of this compound

(0.01 ng: $P = 0.0005$; 100 ng: $P = 0.003$) (Figure 2.2, Appendix D). There was also a significant negative relationship between the compound concentration and the antennal response from the *B. cephi* males ($F = 13.19$, $P = 0.0008$) (Appendix D).

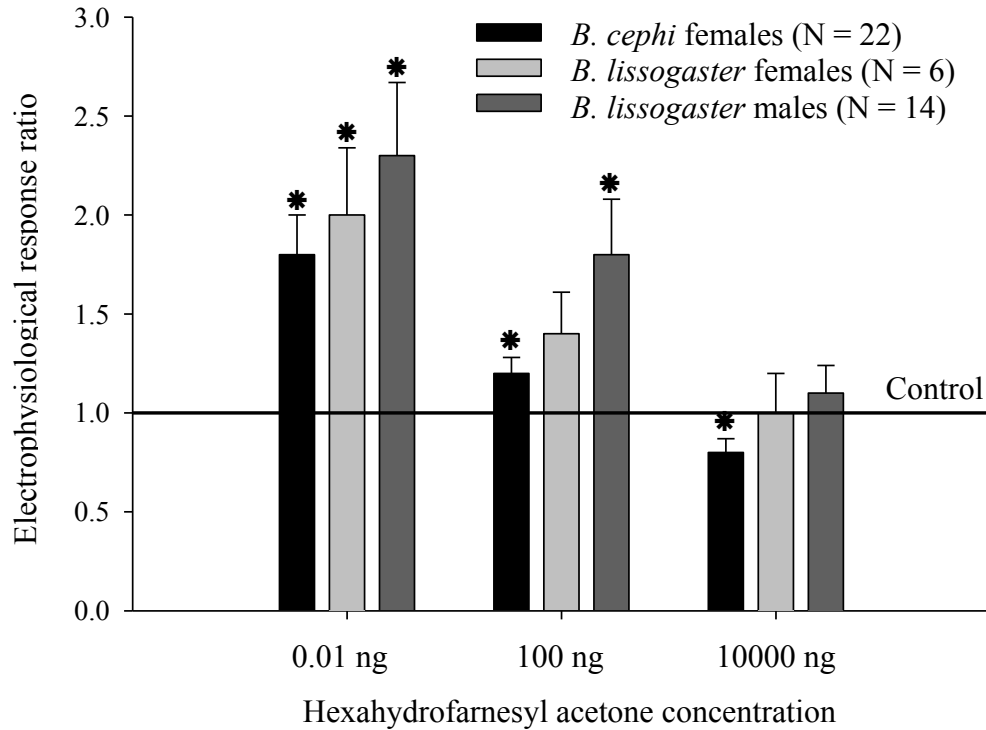


Figure 2.2. Electrophysiological response ratios from the antennae of female *B. cephi* and *B. lissogaster* and male *B. lissogaster* parasitoids to three concentrations of hexahydrofarnesyl acetone. *: Statistically significant ($\alpha = 0.05$).

The average antennal response of *B. cephi* females (1.3 mV) to hexahydrofarnesyl acetone was significantly stronger than the males (0.93 mV) ($t = 2.84$, $P = 0.02$). For the *B. lissogaster* parasitoids, even though the female average antennal response was numerically greater (1.46 mV), there was no significant difference between them and the males (1.22 mV) ($t = 1.34$, $P = 0.19$) (Table 2.1 and 2.2).

There were insufficient male *B. cephi* parasitoids to perform a reliable statistical analysis of their response to this compound (Appendix E).

6-methyl-5-heptene-2-one

A significant response from the antennae of female *B. cephi* parasitoids was produced by the two greatest concentrations (100 and 10000 ng) of this compound ($P = 0.0008$ and < 0.0001) (Appendix B). The largest response ratio (2.0) was generated by the greatest concentration of 6-methyl-5-heptene-2-one (Figure 2.3). There is a positive relationship between the antennal response of these female parasitoids and compound concentration, indicated by an increase in the antennal response ratio as the compound concentration increases ($F = 10.91$, $P = 0.002$).

Female *B. lissogaster* parasitoid antennae produced a significant response only to the greatest concentration of this compound ($P = 0.04$) (Figure 2.3). However, there was a positive, significant relationship between the parasitoid antennal response and the compound concentration ($F = 6.58$, $P = 0.02$) (Appendix C).

The antennae from male *B. lissogaster* parasitoids responded significantly to the two greatest compound concentrations tested ($P = 0.03$ and 0.003) (Appendix D). The largest response ratio of 1.5 was generated by the greatest concentration of 6-methyl-5-heptene-2-one tested (Figure 2.3). The regression analysis showed a positive relationship between the antennal response from the males and the concentrations tested ($F = 11.22$, $P = 0.002$) (Appendix D).

The antennal responses from *B. cephi* parasitoids indicated a significantly stronger response to 6-methyl-5-heptene-2-one for the females (1.54 mV) than for the males

(1.2 mV) ($t = 2.46$, $P = 0.02$) (Table 2.1). The antennal responses from *B. lissogaster* parasitoids was similar, significantly stronger from females (1.75 mV) than males (1.22 mV) ($t = 2.71$, $P = 0.009$) (Table 2.2).

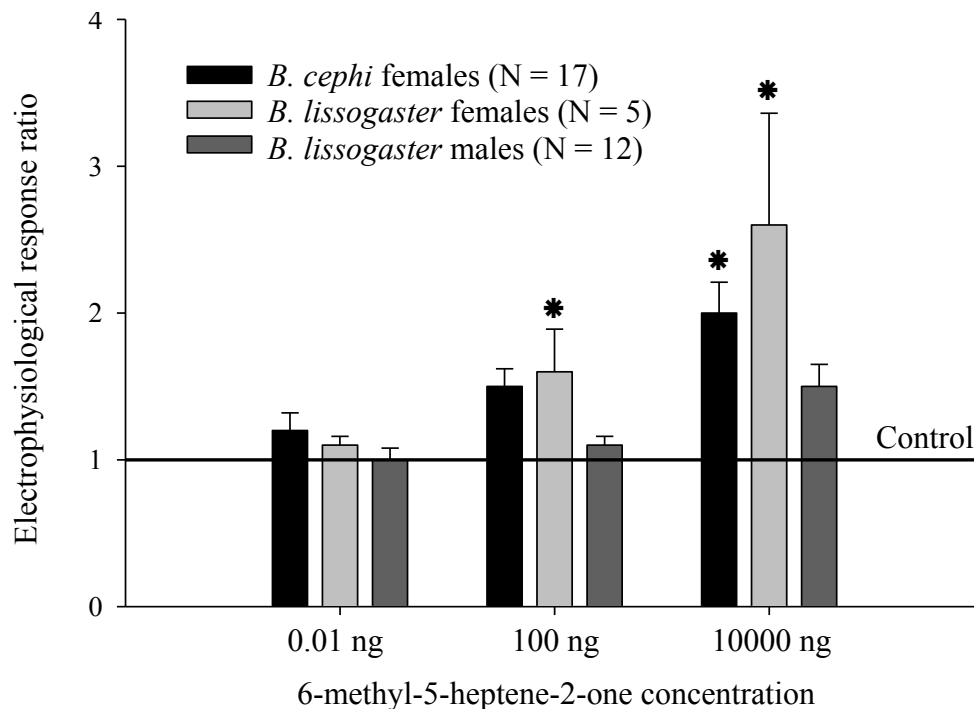


Figure 2.3. Electrophysiological response ratios from the antennae of female *B. cephi* and *B. lissogaster* and male *B. lissogaster* parasitoids to three concentrations of 6-methyl-5-heptene-2-one. *: Statistically significant ($\alpha = 0.05$).

Male *B. cephi* parasitoid antennae did not produce a significant response to any of the tested concentration of this compound and there was no significant relationship between the compound concentration and the antennal response ($F = 5.33$, $P = 0.15$) (Appendix E).

(E)-2-hexenal

For this compound only the greatest concentration tested generated a significant antennal response from males and females of both species (*B. cephi* and *B. lissogaster*)

and from male *B. lissogaster* parasitoids (Females: $P < 0.0001$ and 0.0006 ; Males: $P = 0.002$) (Appendices B, C, and D). The response ratio of 22.6 produced by the concentration of 10000 ng on the antennae of female species was the strongest response obtained from all the tested compounds. There were no significant responses to the lower compound concentrations for any species or sex (Figure 2.4). Regression analysis indicated a significant positive relationship between antennal response and compound concentration for both female and male parasitoids (*B. cephi* females: $F = 37.98$, *B. lissogaster* females: $F = 33.95$, and *B. lissogaster* males: $F = 78.65$; $P < 0.0001$ for all) (Appendices B, C, and D).

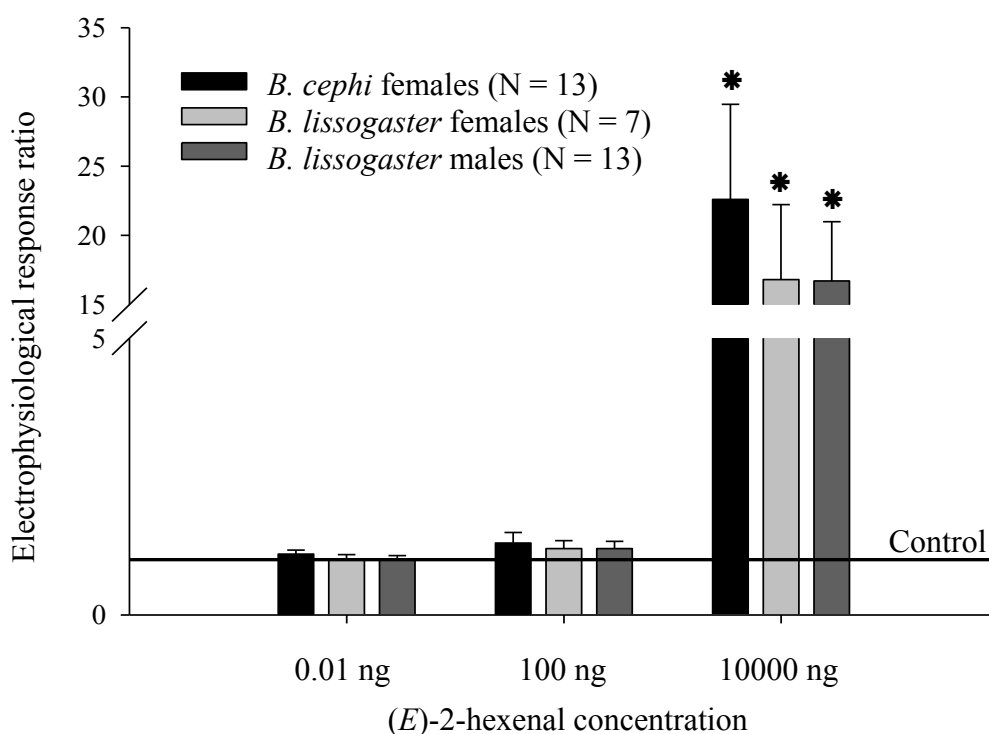


Figure 2.4. Electrophysiological response ratios from the antennae of female *B. cephi* and *B. lissogaster* and male *B. lissogaster* parasitoids to three concentrations of (E)-2-hexenal. * : Statistically significant ($\alpha = 0.05$).

There was a significant difference in the antennal response between female (8.34 mV) and male (1.2 mV) *B. cephi* parasitoids to this compound ($t = 2.58$, $P = 0.01$). For *B. lissogaster* parasitoids, females showed a significantly stronger antennal response (6.3 mV) to this compound, compared to males (1.22 mV) ($t = 2.81$, $P = 0.007$) (Table 2.1 and 2.2).

There were not enough male *B. cephi* parasitoids to perform a reliable statistical analysis of their response to this compound (Appendix E).

(E)-2-hexenyl acetate

Parasitoid antennae only exhibited a significant response to this compound at the largest tested concentration (*B. cephi* females $P = 0.03$, *B. lissogaster* males $P = 0.01$). The lower concentrations did not generate a significant response (Figure 2.5). There was no significant relationship between the antennal responses and the compound concentrations for the *B. cephi* females ($F = 3.42$, $P = 0.07$) (Appendix B). However, *B. lissogaster* males did exhibit a significant positive relationship between the response ratio and the tested concentrations ($F = 9.06$, $P = 0.005$) (Appendix D). There were not sufficient female *B. lissogaster* parasitoids to perform a reliable analysis on this data.

There were no significant differences in the antennal response between female (1.14 mV) and male (1.2 mV) *B. cephi* parasitoid to this compound ($t = 0.36$, $P = 0.72$). Similar results were obtained for *B. lissogaster* parasitoids, there were no significant difference between female (1.5 mV) and male (1 mV) antennal response to this compound ($t = 1.62$, $P = 0.13$) (Table 2.1 and 2.2).

There were not sufficient male *B. cephi* parasitoids to perform a reliable statistical analysis of their response to this compound (Appendix E).

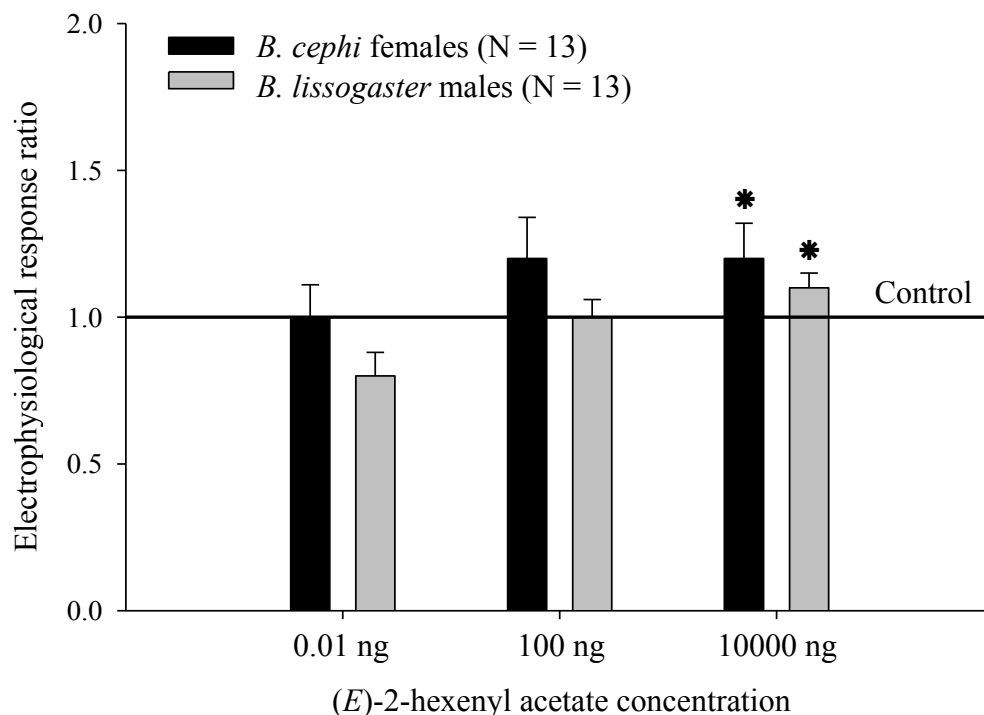


Figure 2.5. Electrophysiological response ratios from the antennae of female *B. cephi* and male *B. lissogaster* parasitoids to three concentrations of (*E*)-2-hexenyl acetate. *: Statistically significant ($\alpha = 0.05$).

Benzaldehyde

This compound only generated a significant response from the antennae of *B. cephi* female parasitoids at the greatest concentration tested ($P = 0.03$). The antennal responses to the two lower concentrations and to all three concentrations by the male *B. lissogaster* were not significant (Appendices B, C, and D). The analysis of the data determined a significant positive relationship between response ratio and compound concentration for *B. cephi* females ($F = 9.20$, $P = 0.005$), but no significant relationship for *B. lissogaster* males ($F = 2.79$, $P = 0.10$) (Appendices B and D). There were not sufficient female *B.*

lissogaster parasitoids to perform a reliable analysis of the antennal response of these parasitoids to the compound concentrations. There was no significant difference between female and male antennal response to this compound for either *B. cephi* ($t = 1.68$, $P = 0.10$) or *B. lissogaster* parasitoids ($t = 0.40$ $P = 0.70$) (Table 2.1 and 2.2).

There were not sufficient male *B. cephi* parasitoids to perform a reliable statistical analysis of their response to this compound (Appendix E).

(Z)-3-hexanol, (α)-pinene, (E)- β -ocimene, and Decanal

These four compounds produced a similar response from the antennae of female *B. cephi* and *B. lissogaster* and from male *B. cephi* and *B. lissogaster* parasitoids. There was no significant response from the antennae of these parasitoids to any concentration of the tested compounds (Appendices B, C, D and E). No significant relationships were found between the response ratio of the parasitoid antennae and the any of the tested concentrations of these compounds (Appendices B, C, D and E). The antennal responses of the female parasitoids of neither *B. cephi* nor *B. lissogaster* were significantly different from those of the males for any of these compounds (Table 2.1 and 2.2).

From the ten wheat compounds that were tested during this study, five compounds generated consistent significant antennal responses from males and females of both of the parasitoid species tested. The compounds (Z)-3-hexenyl acetate, 6-methyl-5-heptene-2-one, (E)-2-hexenal, and (E)-2-hexenyl acetate, all produced a significant response from the parasitoid antennae at the highest concentration tested (10000 ng). In this group of five, only hexahydrofarnesyl acetone did not generate a significant response at the highest concentration tested.

Table 2.1. Antennal responses of female and male *B. cephi* parasitoids to volatile compounds produced by sawfly infested wheat plants

Compound	Average responses (mV)				<i>t</i>	<i>df</i>	<i>P</i>
	females	SE	males	SE			
(<i>Z</i>)-3-hexenyl acetate *	2.76	0.24	1.27	0.12	5.5	54.4	< 0.0001
hexahydrofarnesyl ac. *	1.30	0.09	0.93	0.09	2.84	67	0.02
6-m-5-h-2-o *	1.54	0.10	1.20	0.09	2.46	29.9	0.02
(<i>E</i>)-2-hexenal *	8.34	2.77	1.20	0.09	2.58	38.1	0.01
(<i>E</i>)-2-hexenyl acetate	1.14	0.07	1.20	0.10	0.35	43	0.72
Benzaldehyde	1.21	0.15	0.95	0.08	1.68	34.2	0.10
(<i>Z</i>)-3-hexanol	1.14	0.07	1.03	0.11	0.72	58	0.48
(α)-pinene	0.93	0.06	0.95	0.14	-0.11	34	0.92
(<i>E</i>)- β -ocimene	0.90	0.04	1.00	0.09	-1.22	40	0.23
Decanal	0.99	0.07	0.99	0.09	-0.08	46	0.93

*: Significant ($\alpha = 0.05$); ac.: acetone; 6-m-5-h-2-o: 6-methyl-5-heptene-2-one

Table 2.2. Antennal responses of female and male *B. lissogaster* parasitoids to volatile compounds produced by sawfly infested wheat plants

Compound	Average responses (mV)				<i>t</i>	<i>df</i>	<i>P</i>
	females	SE	males	SE			
(<i>Z</i>)-3-hexenyl acetate *	3.18	0.43	1.33	0.07	4.22	27.5	0.0002
hexahydrofarnesyl ac.	1.46	0.17	1.72	0.18	1.10	50.1	0.28
6-m-5-h-2-o *	1.75	0.28	1.22	0.06	2.71	49	0.009
(<i>E</i>)-2-hexenal	6.30	2.38	6.26	1.83	0.01	58	0.99
(<i>E</i>)-2-hexenyl acetate	1.50	0.31	0.98	0.04	1.62	11.4	0.13
Benzaldehyde	0.97	0.22	1.07	0.06	0.40	12.9	0.70
(<i>Z</i>)-3-hexanol	1.09	0.09	1.11	0.12	0.10	58	0.92
(α)-pinene	0.95	0.07	1.24	0.08	2.04	52	0.05
(<i>E</i>)- β -ocimene	0.98	0.07	0.92	0.05	0.75	58	0.46
Decanal	1.03	0.07	1.02	0.06	0.24	40	0.82

*: Significant ($\alpha = 0.05$); ac.: acetone; 6-m-5-h-2-o: 6-methyl-5-heptene-2-one

Discussion and Conclusions

The consistent significant responses of the parasitoid antennae to (*Z*)-3-hexenyl acetate, 6-methyl-5-heptene-2-one, (*E*)-2-hexenal, (*E*)-2-hexenyl acetate, and hexahydrofarnesyl acetone, suggests a greater sensitivity of *B. cephi* and *B. lissogaster* parasitoid chemoreceptors to these compounds. However, our results revealed an important outcome produced by the variation in the concentrations of some of the tested compounds on the parasitoid antennal responses.

(*Z*)-3-hexenyl acetate

This compound has been found to generate positive electrophysiological and behavioral responses in several insects (Du et al. 1998, Hansson et al. 1999, Reddy and Guerrero 2000, Dickens 2002), including adult females of the host species the wheat stem sawfly (Piesik et al. 2008). The results of our experiments showed that antennae from females of both parasitoid species exhibited a significant response to all three tested concentrations (0.01 ng, 100 ng and 10000 ng) of (*Z*)-3-hexenyl acetate. This suggests the possibility of *B. cephi* and *B. lissogaster* female parasitoids being attracted to this compound and using it to find their hosts. Male *B. lissogaster* antennae also showed a significant response to the two greater concentrations of this compound, although these response ratios were considerably lower. This suggests a greater sensitivity of the chemoreceptors of the female parasitoids to this compound. Greater sensitivity of female antennae to plant compounds is typical (Whitman and Eller 1990, Vaughn et al. 1996, Turlings et al. 2004). It is generally thought that males use the host plant cues that

females orient to for locating mates, while the females are actually seeking acceptable host plants (Engelberth et al. 2004, Zhang and Schlyter 2004, Bruce et al. 2005).

Peck (2004) performed greenhouse studies in which volatiles were collected using a closed volatile system from mechanically wounded wheat plants, mimicking the wounding damage produced by sawfly infestation, which he also tested. His results revealed that there are similar volatile production patterns between wheat plants mechanically wounded and sawfly infested plants. One of the compounds that showed this similar response was (*Z*)-3-hexenyl acetate, which exhibited an increase in its production when the plants were mechanically wounded and when infested by wheat stem sawfly. In addition, the results from the experiments in Chapter 5 (“Influence of wheat stem sawfly infestation level on localized variability of behaviorally active compounds in wheat fields”), determined that wheat plants in areas that are heavily infested by wheat stem sawfly produce greater amounts of (*Z*)-3-hexenyl acetate than those that are much less infested. The strong response of the female antennae of both parasitoid species to the higher concentrations of this compound, and the attractiveness of the parasitoids to this compound, found in behavioral tests presented in Chapter 3, “Behavioral responses of the parasitoids *Bracon cephi* and *Bracon lissogaster* to isolated synthetic volatiles matching those released by wheat plants that are infested by the wheat stem sawfly”, also suggests the possibility of these parasitoids using (*Z*)-3-hexenyl acetate as a chemical cue to find the wheat plants that are infested with wheat stem sawfly.

A stronger antennal response by females of both species of parasitoids to this compound suggest a greater sensitivity of the chemoreceptors on the female antennae to (Z)-3-hexenyl acetate. These results further support the likelihood of the female parasitoids using this compound as a chemical cue to find their host.

Hexahydrofarnesyl acetone

Hexahydrofarnesyl acetone is a sesquiterpene reported to be produced by many different plant species: corn (*Zea mays* L.) (Krokos et al. 2002), ferns (Socolsky et al. 2003), condiment plants (Tümen et al. 1998), strawberries (*Fragaria* spp.) (Khanizadeh and Bélanger 1997), rice (*Oryza sativa* L.) (Miyazawa et al. 2008). It has been shown to induce electrophysiological responses and behavioral activity in several insects: orchid bees (Euglossini) (Eltz et al. 2003, Eltz and Lunau 2005), the European corn borer, *Ostrinia nubilalis* (Hübner) (Binder and Robbins 1997), and the corn stalk borer, *Sesamia nonagrioides* (Lefebvre) (Konstantopoulou et al. 2004). However there is no information currently available on the effect of this compound on the wheat stem sawfly, although it is also a stem boring insect.

Hexahydrofarnesyl acetone is known to be a ripening compound in wheat plants (Miyazawa et al. 2008). Peck (2004) found that the level of this compound in wheat stem sawfly infested wheat plants was lower earlier in the growth stages when the plant was still green and that it increased as the plant ripened. These results were also observed in uninfested plants. Furthermore, results from Chapter 5 (“Influence of wheat stem sawfly infestation level on localized variability of behaviorally active compounds in wheat fields”), corroborated these results. Hexahydrofarnesyl acetone is produced in lower

concentrations when wheat plants are still green and developing and in greater concentrations when wheat plants begin to senesce. These experiments also determined that wheat plants infested with wheat stem sawfly larvae produce significantly lower amounts of this compound compared to uninfested wheat plants, especially during the period when the uninfested wheat plants begin to senesce. Studies by Macedo et al. (2005) found decreased photosynthetic activity wheat plants infested with wheat stem sawfly suggesting an earlier senescence of these plants.

In the wheat stem sawfly system, the braconid parasitoids *B. cephi* and *B. lissogaster* that feed exclusively on wheat stem sawfly larvae have a host searching flight period that extends through plant senescence. During this time, wheat stem sawfly larvae that are inside the wheat stems are in their late developmental instars, making them bigger and more damaging to the wheat plant, but at the same time, a more valuable food source for the parasitoids. The electroantennogram tests that were performed with and female *B. cephi* and *B. lissogaster* parasitoids, revealed strong electrophysiological antennal responses from both male and specifically female parasitoids to low concentrations of hexahydrofarnesyl acetone. Behavioral tests on male and female wheat stem sawfly parasitoids, *B. cephi* and *B. lissogaster*, revealed significant attractiveness to both sexes of these parasitoids to lower concentrations of this ripening compound (Chapter 3: “Behavioral responses of the parasitoids *Bracon cephi* and *Bracon lissogaster* to isolated synthetic volatiles matching those released by wheat plants that are infested by the wheat stem sawfly”).

These results suggest the possibility of these braconid parasitoids using their well developed chemoreceptors to detect the low concentrations of this compound, which will lead them to the wheat plants that are infested with wheat stem sawfly larvae, especially if this difference is a key part of a signature blend of compounds that indicates sawfly infestation. Specifically, female *B. cephi* parasitoids were found to exhibit a stronger antennal response to this compound. However, *B. lissogaster* female and male antennal response showed a similar pattern suggesting the possibility of males using this compound as well to find females.

6-methyl-5-heptene-2-one

In adult female wheat stem sawflies this compound was found to cause repellency (Piesik et al. 2008). Both male and female parasitoids produced positive electrophysiological responses to high concentrations of this compound. Females exhibited particularly strong responses. Behavioral trials in chapter 3 also determined a significant level of attractiveness of the parasitoids to higher concentrations of this compound. This suggests the possibility that this compound might have an important role in the host searching behavior of these braconid parasitoids, probably as another component in a behaviorally active blend.

Based on the significant difference in the antennal response from the female parasitoids of both species, there is a probability of female parasitoid using this compound to detect their hosts feeding inside wheat stems.

(E)-2-hexenal

This aldehyde was not found to be behaviorally attractive to adult wheat stem sawflies in a Y-tube bioassay (Piesik et al. 2008). However, it has been found to generate electrophysiological responses and to be behaviorally attractive to other insects (Guerin et al. 1983, Jönsson and Anderson 1999, Larsson et al. 2001). Our electroantennograph studies showed that this compound generated significant antennal responses from both male *B. lissogaster* and female *B. cephi* and *B. lissogaster* parasitoids. The greatest concentration of (E)-2-hexenal tested was found to produce a very strong antennal response from both males and females of both parasitoid species. However, behavioral tests demonstrated that there was no attractiveness of the parasitoids to high concentrations of this compound (Chapter 3: “Behavioral responses of the parasitoids *Bracon cephi* and *Bracon lissogaster* to isolated synthetic volatiles matching those released by wheat plants that are infested by the wheat stem sawfly”). These results suggest that the olfactory sensitivity of both species of these parasitoids to high concentrations of (E)-2-hexenal is very high, even though it did not generate any observable behavioral response. Because of the magnitude of the response, there is a possibility that an analogue or structural isomer of this compound that fits the neural receptor site of the parasitoids could be a very critical component in host location. The strong response of the parasitoids to a concentration of 10000 ng of this compound suggests the importance of further studies of this compound and those structurally related to it. Tests with different concentrations are also warranted.

(E)-2-hexenyl acetate

This compound was found to produce significant antennal responses from *B. lissogaster* males and *B. cephi* females at the greatest concentration tested. A marginally significant response was also found to be generated on *B. lissogaster* males at the lowest concentration tested. Because behavioral tests results from chapter 3 did not indicate attractiveness to any of the tested parasitoid species, it is unlikely that it is being used in the host searching process. Nevertheless, the positive response produced by high concentration of this compound might suggest its use as a chemical cue at concentrations other than those tested. This compound has been found to be behaviorally attractive to the apple maggot, *Rhagoletis pomonella* (Walsh) (Fein et al. 1982) and it has also been determined to be an inactive compound that synergizes the activity of other compounds (Light et al. 1993).

Benzaldehyde

Benzaldehyde was determined to only generate a significant antennal response from *B. cephi* females at a concentration of 10000 ng. However, it is unlikely that it is being used in the host searching process based on the unattractiveness of the females and males of both parasitoid species to this compound in the behavioral tests performed in Chapter 3. Conversely, the positive response produced by high concentration of the compound might suggest the use of this compound as a chemical cue at other concentrations or it might be used in some other way, perhaps as a synergist. Reddy and Guerrero (2004) provide a list of plant volatiles reported to have synergistic effects and benzaldehyde is mentioned as one of them.

(Z)-3-hexenol, α -pinene, (E)- β -ocimene, and Decanal

These compounds did not produce any significant electrophysiological response from either males or females of both parasitoid species at any of the concentrations tested, so they will not be further assayed. Although these compounds have triggered electrophysiological and behavioral responses in many insect species (Hansson et al. 1999, Jönsson and Anderson 1999, McNair et al. 2000, Larsson et al. 2001, Andersson 2007), including the adult females of the host wheat stem sawfly to (Z)-3-hexenol and (E)- β -ocimene (Piesik et al. 2008), they show no sensory activity at the antennae of either sex of these braconid parasitoids.

In conclusion, there is experimental evidence determining that female insects are generally more sensitive in responding to plant volatile compounds (Jyothi et al. 2002), and males are more responsive to pheromone compounds (Malo et al. 2004). This behavioral response has also been observed in some parasitoid species, *Microplitis croceipes* (Cresson) and *Netelia heroica* Townes (Whitman and Eller 1990), *Cotesia rubecula* (Marshall) (Van Poecke et al. 2001), and *Diaeretiella rapae* (McIntosh) (Vaughn et al. 1996). The results of our experiments partially support this information. Female *B. cephi* and *B. lissogaster* antennae produced stronger electrophysiological responses than males in those compounds that generated a significant response from the parasitoids.

There were five compounds that generated significant responses from the antennae of male and female *B. lissogaster* parasitoids and six compounds from the antennae of *B. cephi* females. The antenna of female parasitoids responded strongly to more compound

concentrations than the male antennae, which supports the idea that their olfactory system is probably more sensitive to these compounds. These data all point towards a quantitatively based blend being used as a potential cue in the host finding by these species. The compounds and concentrations that generated significant responses from the parasitoid antennae will be further studied in a behavioral bioassay using a wind tunnel.

The behavioral analysis will determine which compounds and concentrations are these braconid parasitoids orient towards. More importantly, the type of behavior, specifically attraction, repellency, or indifference will be determined. This information will be very valuable in our efforts to develop an understanding of the atypical host seeking cues used by parasitoids of cryptic stem dwelling insects, and to possibly enhance the efficacy of these biological control agents using information from the chemical ecology of this system.

References

- Agelopoulos, N. G., K. Chamberlain, and J. A. Pickett. 2000. Factors affecting volatile emissions of intact potato plants, *Solanum tuberosum*: variability of quantities and stability of ratios. *J. Chem. Ecol.* 26:497-511.
- Andersson, M. 2007. The effects of non-host volatiles on habitat location by phytophagous insects. Swedish University of Agricultural Sciences, Alnarp, Sweden.
- Baoyu H. and C. Zongmao. 2002. Behavioral and electrophysiological responses of natural enemies to synomones from tea shoots and kairomones from tea aphids, *Toxoptera aurantii*. *J. Chem. Ecol.* 28: 2203-2219.
- Baoyu, H. and H. Baohong. 2007. EAG and behavioral responses of the wingless tea aphid *Toxoptera aurantii* (Homoptera: Aphididae) to tea plant volatiles. *Acta Ecol. Sinica* 27: 4485-4490.
- Binder, B. F., and J. C. Robbins. 1997. Effect of terpenoids and related compounds on the oviposition behavior of the european corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *J. Agric. Food Chem.* 45: 980-984.
- Birkett, M. A., T. J. A. Bruce, J. L. Martin, L. E. Smart, J. Oakley, and L. J. Wadhams. 2004. Responses of female orange wheat blossom midge, *Sitodiplosis mosellana*, to wheat panicle volatiles. *J. Chem. Ecol.* 30: 1319-1328.
- Bjostad, L. B. 1998. Electrophysiological methods. In J. G. Millar and K. F. Haynes (eds): *Methods in chemical ecology*. Chemical methods. Vol. 1. pp. 339-375. Kluwer Academic Publishers. Norwell, Massachusetts.
- Bruce, T. J. and A. Cork. 2001. Electrophysiological and behavioral responses of female *Helicoverpa armigera* to compounds identified in flowers of African marigold, *Tagetes erecta*. *J. Chem. Ecol.* 27: 1119-1131.
- Bruce, T. J. A., L. J. Wadhams and C. M. Woodcock. 2005. Insect host location: a volatile situation. *Trends Plant Sci.* 10: 269-274.
- Buteler, M., D. K. Weaver, and P. R. Miller. 2008. Wheat stem sawfly-infested plants benefit from parasitism of the herbivorous larvae. *Agric. For. Entomol.* 10: 347-354.
- Buttery, R. G., C. Xu, and L. C. Ling. 1985. Volatile components of wheat leaves (and stems): possible insect attractants. *J. Agri. and Food Chem.* 33: 115-117.

- Byers, J. A. 1989. Chemical ecology of bark beetles. *Cell. Mol. Life Sci.* 45: 271-283.
- Byers, J. A., Q. H. Zhang, and F. Schlyter. 1998. Volatiles from nonhost birch trees inhibit pheromone response in spruce bark beetles. *Naturwissenschaften* 85: 557-561.
- Chapman, R. F. 1998. The insects. Structure and function. 4th edition, Cambridge University Press, Cambridge, United Kingdom. pp.770.
- Coleman, R. A., Barker A. M., M. Fenner, and F. C. King. 1997. Relative effect of different host feeding site on long-range host location and electroantennogram response in the parasitoid *Cotesia glomerata* (Hym., Braconidae). *J. Appl. Entomol.* 121: 487-494.
- Cortesero, A. M., J. O. Stapel, and W. J. Lewis. 2000. Understanding and manipulating plant attributes to enhance biological control. *Biol. Control* 17: 34-49.
- De Moraes, C. M., W. J. Lewis, P. W. Paré, H. T. Alborn, and J. H. Tumlinson. 1999. Herbivore-infested plants selectively attract parasitoids. *Nature* 393: 570-573.
- Degenhardt, J. 2009. Indirect defense response to herbivory in grasses. *Plant Physiol.* 149: 96-102.
- Dicke, M. and J. A. J. van Loon. 2000. Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomol. Exp. Appl.* 97: 237-249.
- Dickens, J. C. 1984. Olfaction in the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae): electroantennogram studies. *J. Chem. Ecol.* 10: 1759-1785.
- Dickens, J. C. 2002. Behavioral responses of larvae of Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), to host plant volatile blends attractive to adults. *Agric. For. Entomol.* 4: 309-314.
- Du, Y., G. M. Poppy, W. Powell, J. A. Pickett, L. J. Wadhams, and C. M. Woodcock. 1998. Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. *J. Chem. Ecol.* 24: 1355-1368.
- Dudareva, N., E. Pichersky, and J. Gershenzon. 2004. Biochemistry of plant volatiles. *Plant Physiol.* 135: 1893-1902.

- Eller, F. J., J. H. Tumlinson, and W. J. Lewis. 1988. 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). *Environ. Entomol.* 17: 745-753.
- Eltz, T. and K. Lunau. 2005. Antennal response to fragrance compounds in male orchid bees. *Chemoecology* 15:135-138.
- Eltz, T., D. W. Roubik, and M. W. Whitten. 2003. Fragrances, male display and mating behaviour of *Euglossa hemichlora*: a flight cage experiment. *Physiol. Entomol.* 28: 251-260.
- Engelberth, J., H. T. Alborn, E. A. Schmelz, and J. H. Tumlinson. 2004. Airborne signals prime plants against insect herbivore attack. *P. Natl. Acad. Sci.* 101: 1781-1785.
- Fein, B. I., W. H. Reissig, and W. L. Roelofs. 1982. Identification of apple volatiles attractive to the apple maggot, *Rhagoletis pomonella*. *J. Chem. Ecol.* 8: 1437-1487.
- Foster, S. P. and M. O. Harris. 1997. Behavioral manipulation methods for insect pest-management. *Annu. Rev. Entomol.* 42: 123-146.
- Gouinguené, S. P. and T. C. J. Turlings. 2002. The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiol.* 129: 1296-1307.
- Gouinguené, S., J. A. Pickett, L. J. Wadhams, M. A. Birkett, and T. C. J. Turlings. 2005. Antennal electrophysiological responses of three parasitic wasps to caterpillar-induced volatiles from maize (*Zea mays mays*), cotton (*Gossypium herbaceum*), and cowpea (*Vigna unguiculata*). *J. Chem. Ecol.* 31: 1023-1028.
- Guerin, P. M., E. Städler, and H. R. Buser. 1983. Identification of host plant attractants for the carrot fly, *Psila rosae*. *J. Chem. Ecol.* 9: 843-861.
- Hamilton-Kemp, T. R., and R. A. Andersen. 1984. Volatile compounds from *Triticum aestivum*. *Phytochemistry.* 23: 1176-1177.
- Hansson, B., M. C. Larsson, and W. S. Leal. 1999. Green leaf volatile-detecting olfactory receptor neurones display very high sensitivity and specificity in a scarab beetle. *Physiol. Entomol.* 24: 121-126.
- Hartel, K. D., B. A. Frederick, and T. G. Shanower. 2003. Isolation and characterization of microsatellite loci in wheat stem sawfly *Cephus cinctus* and cross-species amplification in related species. *Molec. Ecol. Notes.* pp. 1-3.

- Hibbard, B. E., T. L., Randolph, E. J. Bernklau, E. M. Abou-Fakhr, and L. B. Bjostad. 1997. Electroantennogram-active components of maize silk for adults of the western corn rootworm (Coleoptera: Chrysomelidae). *Environ. Entomol.* 26: 285-296.
- James, D. G. 2003. Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: Methyl salicylate and the green lacewing, *Chrysopa nigricornis*. *J. Chem. Ecol.* 29: 1601-1609.
- Janssen, A., J. Bruin, G. Jacobs, R. Schraag, and M. Sabelis. 1997. Predators use volatiles to avoid prey patches with conspecifics. *J. Anim. Ecol.* 66: 223-232.
- Jönsson, M., and P. Anderson. 1999. Electrophysiological response to herbivore-induced host plant volatiles in the moth *Spodoptera littoralis*. *Physiol. Entomol.* 24: 377-385.
- Jyothi, K. N., A. L. Prasuna, S. Sighamony, B. K. Kumari, A. R. Prasad and J. S. Yadav. 2002. Electroantennogram responses of *Apanteles obliquae* (Hym., Braconidae) to various infochemicals. *J. Appl. Entomol.* 126: 175-181.
- Kennedy, G. 2003. Tomato, pests, parasitoids, and predators: Tritrophic interactions involving the genus *Lycopersicon*. *Annu. Rev. Entomol.* 48:51-72.
- Kessler, A., and T. Baldwin. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291: 2141-2144.
- 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 Manag. Sci.* 56:957-962.
- Khanizadeh, S. and Bélanger A, 1997. Classification of 92 strawberry genotypes based on their leaf essential oil composition. *Acta Hort.* 439: 205-209.
- Konstantopoulou, M. A., F. D. Krokos, and B. E. Mazomenos. 2004. Chemical composition of corn leaf essential oils and their role in the oviposition behavior of *Sesamia nonagrioides* females. *J. Chem. Ecol.* 30: 2243-2256.
- Koschier, E. H. and K. A. Sedy. 2001. Effects of plant volatiles on the feeding and oviposition of *Thrips tabaci*. In: R. Marullo and L. A. Mound (eds): *Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera*. pp. 185-187. Reggio, Calabria, Italy, July 2-7, 2001.

- Kozlowski, M. W. and J. H. Visser. 1981. Host plant related properties of the antennal olfactory system in the oak flea weevil, *Rhynchaenus quercus* - electroantennogram study. *Entomol. Exp. Appl.* 30: 169-175.
- Krokos, F. D., M. A. Konstantopoulou, and B. E. Mazomenos. 2002. Chemical characterisation of corn plant compounds by different extraction techniques and the role of potent chemicals in the reproductive behavior of the corn stalk borer *Sesamia nonagrioides*. *IOBC wprs Bull.* 25: 1-9.
- Landolt, P. J. and T. W. Phillips. 1997. Host plant influences on sex pheromone behavior of phytophagous insects. *Annu. Rev. Entomol.* 42: 371-391.
- Larsson, M. C., W. S. Leal, and B. S. Hansson. 2001. Olfactory receptor neurons detecting plant odours and male volatiles in *Anomala cuprea* beetles (Coleoptera: Scarabaeidae). *J. Insect Physiol.* 47: 1065-1076.
- Lewis, W. J., D. A. Nordlund, R. C. Gueldner, P. E. Teal, and J. H. Tumlinson. 1982. Kairomones and their use for management of entomophagous insects. XIII. Kairomonal activity for *Trichogramma* spp. of abdominal tips, excretions, and a synthetic sex pheromone blend of *Heliothis zea* (Boddie) moths. *J. Chem. Ecol.* 8: 1323-1331.
- Lewis, W. J., W. Sheehan, and J. H. Tumlinson. 1994. Unraveling the story of how parasitoids find their host. *In* Pest Management in the subtropics. Biological control - a Florida perspective. Intercept Ltd. Andover, Hampshire. 520 p.
- Light, D. M., R. A. Flath, R. G. Buttery, F. G. Zalom, and R. E. Rice. 1993. Host-plant green-leaf volatiles synergize the synthetic sex pheromones of the corn earworm and codling moth (Lepidoptera). *Chemoecology* 4: 145-152.
- Macedo, T. B., R. K. D. Peterson, D. K. Weaver, and W. L. Morrill. 2005. Wheat stem sawfly, *Cephus cinctus* Norton, impact on wheat primary metabolism: An ecophysiological approach. *Environ. Entomol.* 34: 719-726.
- Malo, E. A., V. R. Castrejo, N. Gomez, L. Cruz-Lopez, and J. C. Rojas. 2004. Antennal sensilla and electrophysiological response of male and female *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to conspecifics sex pheromone and plant odors. *Ann. Entomol. Soc. Am.* 97: 1273-1284.
- McNair, C., G. Gries, and R. Gries. 2000. Cherry bark tortrix, *Enarmonia formosana*: Olfactory recognition of and behavioral deterrence by nonhost angio- and gymnosperm volatiles. *J. Chem. Ecol.* 26: 809-821.

- 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.
- Morrill, W. L. and G. D. Kushnak. 1996. Wheat stem sawfly (Hymenoptera: Cephidae) adaptation to winter wheat. *Environ. Entomol.* 25: 1128-1132.
- Miyazawa, M., S. Nagai, and T. Oshima. 2008. Volatile components of the straw of *Oryza sativa* L. *J. Oleo Sci.* 57: 139-144.
- Ode, P. J. 2006. Plant chemistry and natural enemy fitness: Effects on herbivore and natural enemy interactions. *Annu. Rev. Entomol.* 51:163-85.
- Ott, L. 1993. An introduction to statistical methods and data analysis. 4th edition, Duxbury Press, Belmont, California. pp 1051.
- Peck, G. E. 2004. Potential semiochemicals of wheat (*Triticum aestivum* L.) induced by oviposition and feeding of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). [Master's Thesis]. Entomology, Montana State University, Bozeman, Montana. 65 p.
- Pickett, J. A., L. J. Wadhams, C. M. Woodcock, and J. Hardie. 1992. The chemical ecology of aphids. *Annu. Rev. Entomol.* 37: 67-90.
- Pickett, J. A., L. J. Wadhams, and C. M. Woodcock. 1997. Developing sustainable pest control from chemical ecology. *Agric. Ecosyst. Environ.* 64: 149-156.
- Piesik, D., D. K. Weaver, J. B. Runyon, M. Buteler, G. E. Peck, and W. L. Morrill. 2008. Behavioral responses of wheat stem sawflies to wheat volatiles. *Agric. For. Entomol.* 10: 245-253.
- Quinn, G. P. and M. J. Keough. 2002. Experimental design and data analysis for biologist. Cambridge University Press, United Kingdom. 537 p.
- Randlkofer, B., E. Obermaier, and T. Meiners. 2007. Mother's choice of the oviposition site: balancing risk of egg parasitism and need of food supply for the progeny with an infochemical shelter? *Chemoecology* 17: 177-186.
- Reddy, G. V. P. and A. Guerrero. 2000. Behavioral responses of the diamondback moth, *Plutella xylostella*, to green leaf volatiles of *Brassica oleracea* Subsp. *capitata*. *J. Agric. Food Chem.* 48: 6025-6029.
- Reddy, G. V. P. and A. Guerrero. 2004. Interactions of insect pheromones and plant semiochemicals. *Trends Plant Sci.* 9: 253-261.

- Rojas, J. C. 1999. Electrophysiological and behavioral responses of the cabbage moth to plant volatiles. *J. Chem. Ecol.* 25: 1867-1883.
- Rott, A. S., J. Häckermann, N. Brand, A. Vallat, and S. Dorn. 2005. Parasitoid exploitation of the seasonal variation in host plant volatile emission for herbivore location. *Entomol. Exp. Appl.* 115: 199-205.
- Ryan, M. F. 2002. Insect chemoreception. Fundamental and Applied. Kluwer Academic Publishers, Dordrecht, The Netherlands. 330 p.
- Salom, S. M., I. G. Birgersson, T. L. Payne, and C. W. Berisford. 1991. Electroantennogram responses of the southern pine beetle parasitoid *Dinotiscus dendroctoni* (Ashmead) (Hymenoptera: Pteromalidae) to potential semiochemicals. *J. Chem. Ecol.* 17: 2527-2538.
- Schneider, D. 1957. Elektrophysiologische untersuchungen von chemo- und mechanorezeptoren der antenne des seidenspinners *Bombyx mori* L. *Z. Vergl. Physiol.* 40: 8-41.
- Socolsky, C., A. Salvatore, Y. Asakawa, and A. Bardón. 2003. Bioactive new bitter-tasting *p*-hydroxystyrene glycoside and other constituents from the fern *Elaphoglossum spathulatum*. *Arkivoc*: 347-355.
- Syntech. 1998. Electroantennography. A practical introduction. Syntech, Hilversum, The Netherlands. 14 p.
- Tentelier, C. and X. Fauvergue. 2007. Herbivore-induced plant volatiles as cues for habitat assessment by a foraging parasitoid. *J. Anim. Ecol.* 76: 1-8.
- Thiéry, D. and F. Marion-Poll. 1998. Electroantennogram responses of Douglas-fir seed chalcids to plant volatiles. *J. Insect Physiol.* 44: 483-490.
- Tümen, G., K. H. C. Başer, B. Demirci and N. Ermin. 1998. The essential oils of *Satureja coerulea* Janka and *Thymus aznavourii* Velen. *Flavour Fragr. J.* 13: 65-67.
- Tumlinson, J. H., W. J. Lewis, and E. M. Louise. 1993. How parasitic wasps find their hosts. *Sci. Am.* 264: 100-106.
- Turlings, T. C., J. H. Tumlinson, and W. J. Lewis. 1990. Exploitation of herbivore induced plant odors by host-seeking parasitic wasps. *Science* 250: 1251-1253.

- Turlings, T. C., J. H. Loughrin, P. J. McCall, U. S. R. Röse, W. J. Lewis, and J. H. Tumlinson. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proc. Natl. Acad. Sci. USA*. 92: 4169-4174.
- Turlings, T. C. J., M. Bernasconi, R. Bertossa, F. Bigler, G. Caloz, and S. Dorn. 1998. The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. *Biol. Control* 11:122-129.
- Van Poecke, R. M. P., Maarten A. Posthumus, M. A., and M. Dicke. 2001. Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: chemical, behavioral, and gene-expression analysis. *J. Chem. Ecol.* 27: 1911-1928.
- Vaughn, T. T., M. F. Antolin, and L. B. Bjostad. 1996. Behavioral and physiological responses of *Diaeretiella rapae* to semiochemicals. *Entomolo. Exper. Applic.* 78: 187-196.
- Weaver, D. K., S. E. Sing, J. B. Runyon, and W. L. Morrill. 2004. Potential impact of cultural practices on wheat stem sawfly (Hymenoptera: Cephidae) and associated parasitoids. *J. Agric. Urban. Entomol.* 21: 271-287.
- 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. *Biol. Control* 34: 1-11.
- Weiss, M. J. and W. L. Morrill. 1992. Wheat stem sawfly (Hymenoptera: Cephidae) revisited. *Am. Entomol.* 38: 241-245.
- Whitman, D. W. and F. J. Eller. 1990. Parasitic wasps orient to green leaf volatiles. *Chemoecology* 1: 69-71.
- Zhang, Q. H. and F. Schlyter. 2004. Olfactory recognition and behavioral avoidance of angiosperm nonhost volatiles by conifer-inhabiting bark beetles. *Agri. For. Entomol.* 4: 1-19.

CHAPTER 3

BEHAVIORAL RESPONSES OF THE PARASITOIDS *BRACON CEPHI* AND
BRACON LISSOGASTER TO ISOLATED SYNTHETIC VOLATILES
MATCHING THOSE RELEASED BY WHEAT PLANTS THAT
ARE INFESTED BY THE WHEAT STEM SAWFLY

Introduction

The wheat stem sawfly *Cephus cinctus* Norton (Hymenoptera: Cephidae), a stem-boring species (Ainslie 1920, Farstad 1940) that is one of the most serious insect pests of wheat (*Triticum aestivum* L.) in the Northern Great Plains of North America, causes tens of millions of dollars in losses each year (Weiss and Morrill 1992, Weaver et al. 2004, 2005). This insect, which modified its feeding habit from naturally occurring to cultivated grasses (Callenbach and Hansmeier 1945, Holmes 1978), has one generation per year. Adult sawflies mate after they emerge in late spring and immediately begin to lay eggs inside the stems of wheat plants. The larval stage of this pest is spent entirely inside the wheat stem where it feeds on the parenchymous and vascular tissue of the plant (Holmes 1954, 1977).

The cryptic nature of the wheat stem sawfly, living most of its life inside the wheat stem, has made it a very difficult pest to control. Currently, there is no single effective method to control this pest, only partial control has been obtained by combining different cultural techniques, and primary among these is the use of solid-stemmed wheat varieties. There is a great necessity to find an effective control method for this pest.

The implementation of chemical ecology in the control of pests has demonstrated to be effective (Foster and Harris 1997, Pickett et al. 1997, Agelopoulos et al. 1999). Plants produce many different kinds of volatile compounds and these serve a variety of different functions within the plant (Dudareva et al. 2004, Goff and Klee 2006, Pichersky et al. 2006). Some volatiles are involved in plant communication (Pichersky and Gang 2000, Holopainen 2004, Heil et al. 2008), others are produced as defensive mechanisms against pests (Paré and Tumlinson 1999, Kessler and Baldwin 2001, Sabelis et al. 2001, James 2003, Bruce et al. 2005), and there are numerous volatiles produced for no apparent reason (Peñuelas and Llusà 2004).

The understanding of the chemical ecology involved in the use of semiochemicals by plants as direct or indirect defensive mechanisms is very important toward the implementation of this technique in managing pests of different crops. There is consistent information on the production of volatile compounds by plants as a response to the attack by herbivorous pests (Bernasconi et al. 1998, Dorn and Hern 1999, Peck 2004, Ode 2006, Degenhardt 2009). The study of plant defensive mechanisms has demonstrated that they are capable of producing specific compounds, blend of compounds, or to modify the amount of volatile compounds produced that attract natural enemies (Cortesero et al. 2000, James 2003, D'Alessandro & Turlings 2005). Within the natural enemies that plants attract, parasitoids are one of the most important groups. There are many examples of plants under attack by herbivore pests producing volatiles that attract parasitoids (Turlings et al. 1990, Tumlinson et al. 1993, De Moraes et al. 1998, Du et al. 1998, Pettersson and Boland 2003, Rott et al. 2005, Agbogba & Powell 2007).

Within the wheat stem sawfly system two braconid parasitoids, *B. cephi* (Gahan) and *B. lissogaster* Muesebeck (Hymenoptera: Braconidae) have been found to be important natural regulators of sawfly populations in wheat (Holmes et al. 1963, Morrill et al. 1994, Runyon et al. 2002, Weaver et al. 2005). In order for parasitoids to be efficient biological control agents, they must have good host-seeking abilities. Host seeking by larval parasitoids attacking the cryptic wheat stem sawfly is an example of a system that is most likely to require distinct chemical cues because parasitoids are already known to use herbivore-induced plant odors to detect their hosts (Vinson 1976, Turlings et al. 1990, Tumlinson et al. 1993, Paré and Tumlinson 1999, Agbogba and Powell 2007), but the body of literature is dominated by parasitoids of external foliar feeding species that can also be seen by foraging females (Mattiacci et al. 1995, Du et al. 1998, Turlings et al. 1998, Lo Pinto et al. 2003). In fact, the lack of unique induced compounds by insects with stem boring habits has limited research in this area (Ngi-Song and Overholt 1997, Turlings et al. 1998, Tooker and De Moraes 2007), given the paradigm established by the work on foliar feeding species and has perhaps led to incorrect conclusions due to methodological constraints (Tooker and De Moraes 2007). Finding behaviorally active compounds for parasitoids that are collected from wheat stems will address potential host seeking strategies, even in the absence of unique signaling molecules. In addition, the possibility of finding a method to enhance host-seeking by parasitoids using chemical cues would constitute an important tool in the control of pests as has been shown for other systems (Pickett et al. 1997, Agelopoulos et al. 1999, Cortesero et al. 2000).

Semiochemicals produced by sawfly-infested wheat plants have been found to generate electrophysiological responses in male and female parasitoids (Chapter 2, “Electroantennogram responses of parasitoids *Bracon cephi* and *Bracon lissogaster* to synthetic wheat volatiles matching those released by wheat plants infested with wheat stem sawfly”). This study assesses the behavioral responses of the parasitoids *B. cephi* and *B. lissogaster* to these compounds using a choice test bioassay in a wind tunnel. The wind tunnel technique that is used to determine behavioral responses from insects has been used effectively with other parasitoids (Elzen et al. 1986, McCall et al. 1993, Fatouros et al. 2005, Ichiki et al. 2008, Williams III et al. 2008).

The results will take us a step closer in determining the possibility of understanding host seeking by these parasitoids, and introduces the possibility of using active compounds that are identified as tools to enhance the efficiency of these parasitoids in the control of the wheat stem sawfly. Specifically, if it is possible to find compounds that attract the parasitoids to the sawfly larvae inhabiting wheat plants, it might be possible to augment the quantities of these compounds to control this pest (Agelopoulos et al. 1999, Cortesero et al. 2000).

Objective

Determine if there is a behavioral response from *B. cephi* and *B. lissogaster* parasitoids to isolated, synthetic volatiles matching those produced by wheat plants infested with the wheat stem sawfly, using compounds that generated a positive electrophysiological response from the parasitoid antennae.

Test Hypothesis

The synthetic volatiles matching those produced by sawfly infested wheat plants that generated a positive electrophysiological response from the parasitoid antennae will produce a significant behavioral response from *B. cephi* and *B. lissogaster* parasitoids.

Materials and Methods

Insect Material

The parasitoids used in the experiment were collected during the fall from postharvest wheat stubble in Montana. The collected wheat stubble was used to obtain parasitoids by two different methods which were described in Chapter 2 (“Electroantennogram responses of parasitoids *Bracon cephi* and *Bracon lissogaster* to synthetic wheat volatiles matching those released by wheat plants infested with wheat stem sawfly”).

Wind Tunnel System

The wind tunnel used in these behavioral experiments consisted of a rectangular acrylic box 150 cm long, 50 cm wide, and 50 cm high. The airflow rate used inside the wind tunnel during the experiments was 0.2 liters per minute.

Two plastic Petri dishes (Fisher Scientific, Pittsburgh, Pennsylvania) each with a 2 x 2 cm square hole in the center and with the outside surface covered with Tanglefoot[®] were placed at the end of the wind tunnel at a height of 20 cm and separated 30 cm from each other (Figure 3.1). Each Petri dish had a paper clip holding a treated rubber septum

in the center of the square hole. The rubber septum would emanate the compound that had been applied to it into the airstream. Positive phototaxis was used by placing a 12 V light bulb behind each hole of the Petri dishes. This will encourage the movement of the parasitoids toward the Petri dishes. The wind tunnel was placed inside a dark room with a controlled temperature of 27°C.



Figure 3.1. Wind tunnel apparatus used to test behavioral responses of *B. cephi* and *B. lissogaster* to synthetic compounds known to be produced by wheat plants infested by wheat stem sawfly.

Behavioral Experiments

Pure synthetic compounds representing those that are produced by wheat plants infested by wheat stem sawflies were used to perform the behavior trials. The compounds were the same ones tested in the electrophysiology experiments in Chapter 2, “Electroantennogram responses of parasitoids *Bracon cephi* and *Bracon lissogaster* to

synthetic wheat volatiles matching those released by wheat plants infested with wheat stem sawfly”). These highly pure compounds were diluted with hexane (Certified A.C.S. Spectranalyzed[®], Fisher Scientific, New Jersey) to obtain the concentrations to be tested in the behavior bioassays with the braconid parasitoids. The compounds and amounts that were applied in a 10 µl aliquot are: (Z)-3-hexenyl acetate (Bedoukian Research Inc., Danbury, CT): 90 µg, 6-methyl-5-heptene-2-one (99%, Aldrich Chem. Co., Milwaukee, Wisconsin): 35 µg, hexahydrofarnesyl acetone (low) (Bedoukian Research Inc., Danbury, CT): 0.3 ng, hexahydrofarnesyl acetone (high): 9 µg, (Z)-3-hexanol (98%, Aldrich Chem. Co., Milwaukee, Wisconsin): 423 µg, (E)-2-hexenyl acetate (98%, Aldrich Chem. Co., Milwaukee, Wisconsin): 1.4 µg, (E)-2-hexenal (99%, Aldrich Chem. Co., Milwaukee, Wisconsin): 423 µg, (E)- β -ocimene (70%, Fluka Chemika, Aldrich Chem. Co., Milwaukee, Wisconsin): 215 µg.

Some of the concentrations used in this bioassay were selected from the work of Piesik et al. (2008). In this study, behavioral tests were performed with wheat stem sawfly adults using volatile compounds matching those released by wheat plants. The range of concentrations used in their experiments was selected based on the mean amounts of these compounds that were collected from individual wheat plants over a 10 hr period. Other compound concentrations were selected based on sensory tests conducted with the braconid parasitoids (Chapter 2, “Electroantennogram responses of parasitoids *Bracon cephi* and *Bracon lissogaster* to synthetic wheat volatiles matching those released by wheat plants infested with wheat stem sawfly”) and subsequent

preliminary behavioral tests spanning a broad range of ten-fold increasing concentrations to determine target amounts for a rigorous bioassay.

For each compound tested in these trials, 100 female and 100 male parasitoids were used in groups of 10 at a time (Appendix F). For each replication, 10 female or male parasitoids were placed on a 25 cm high platform at a distance of 100 cm from the odor source. A total of 10 replications were performed for each compound concentration and for each sex. Because the availability of these parasitoid species during the experimental trials was occasionally scarce, it was sometimes necessary to combine both species for behavior tests. These species are spatially and temporally sympatric co-occurring in nature and both are host specific. Accumulation of a standard number of individuals within a species was not possible nor did it seem appropriate to vary the test numbers. For each tested compound, 10 μ l of test solution was randomly applied to one of the two septa on the Petri dishes; the other one was treated with 10 μ l of hexane (Certified A.C.S. Spectranalyzed[®], Fisher Scientific, New Jersey) as a control. Parasitoids were left for 24 hours inside the wind tunnel for each replication. The initial parasitoid response was assessed after the first 8 hours of the test by recording the number of parasitoids captured in each Petri dish. After 24 hours a final parasitoid count was recorded.

After each replication, both septa were discarded and replaced with new ones. After each tested compound both Petri dishes were replaced with new ones. Petri dish positions were randomly exchanged over the replications that were run for each compound.

Statistical Analysis

The data collected from the behavioral trials consisted of number of insects captured. Only data where all captured insects were a single species and the number of individuals captured by both traps was greater than 4 were used. This data was transformed into a percentage of parasitoid capture on the treated or control trap, which represents the responses of the parasitoid species to the compounds. The percentage data was transformed using an arcsine transformation and analyzed using a t-test to determine if there were statistically significant differences between the treatments and controls for each tested compound. Comparisons between male and female behavioral responses to each tested compound were analyzed using a t-test. The analysis was performed in SAS (Version 9.2, SAS Institute, 2007). The percentage response (parasitoid capture) to each compound was plotted with the associated standard error (SE).

Results

Behavioral tests revealed that the parasitoids from both species were found to be significantly attracted to (Z)-3-hexenyl acetate applied at 90 µg in a wind tunnel bioassay (Figure 3.2). Female *B. cephi* parasitoids (63%) exhibited the strongest attraction to this compound ($P < 0.0001$). Male *B. lissogaster* parasitoids (57%) were also found to be significantly attracted to this compound ($P < 0.0001$) and the females (53%) showed a marginally significant attraction as well ($P = 0.05$) (Tables 3.1, 3.2 and 3.3).

Comparison of the behavioral responses between female and male *B. lissogaster* parasitoids determined a significantly stronger response from the females to (Z)-3-hexenyl acetate ($t = 1.17$, $P = 0.02$) (Table 3.4).

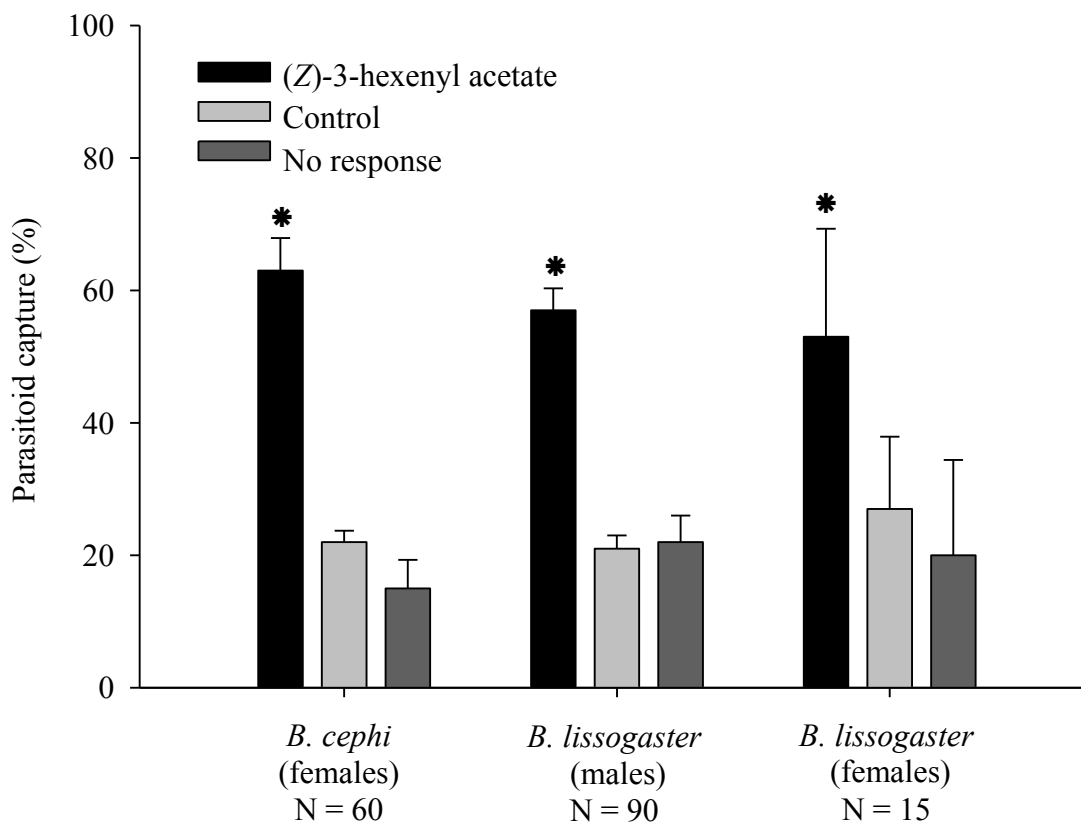


Figure 3.2. Parasitoid behavioral responses to (Z)-3-hexenyl acetate (90 μ g) in a wind tunnel. *: Statistically significant ($\alpha = 0.05$).

The electrophysiologically active compound 6-methyl-5-heptene-2-one was also significantly attractive to both braconid parasitoid species (Figure 3.3). Female *B. lissogaster* parasitoids (61%) were highly attracted to this compound ($P < 0.0001$) (Table 3.3). Both *B. cephi* females (58%) and *B. lissogaster* males (47%) showed significant attraction to 6-methyl-5-heptene-2-one as well ($P < 0.0001$) (Tables 3.1 and 3.2).

There was a significantly stronger response from female *B. lissogaster* parasitoids (74%) to this compound than from the males (61%) ($t = 3.22$, $P = 0.007$) (Table 3.4).

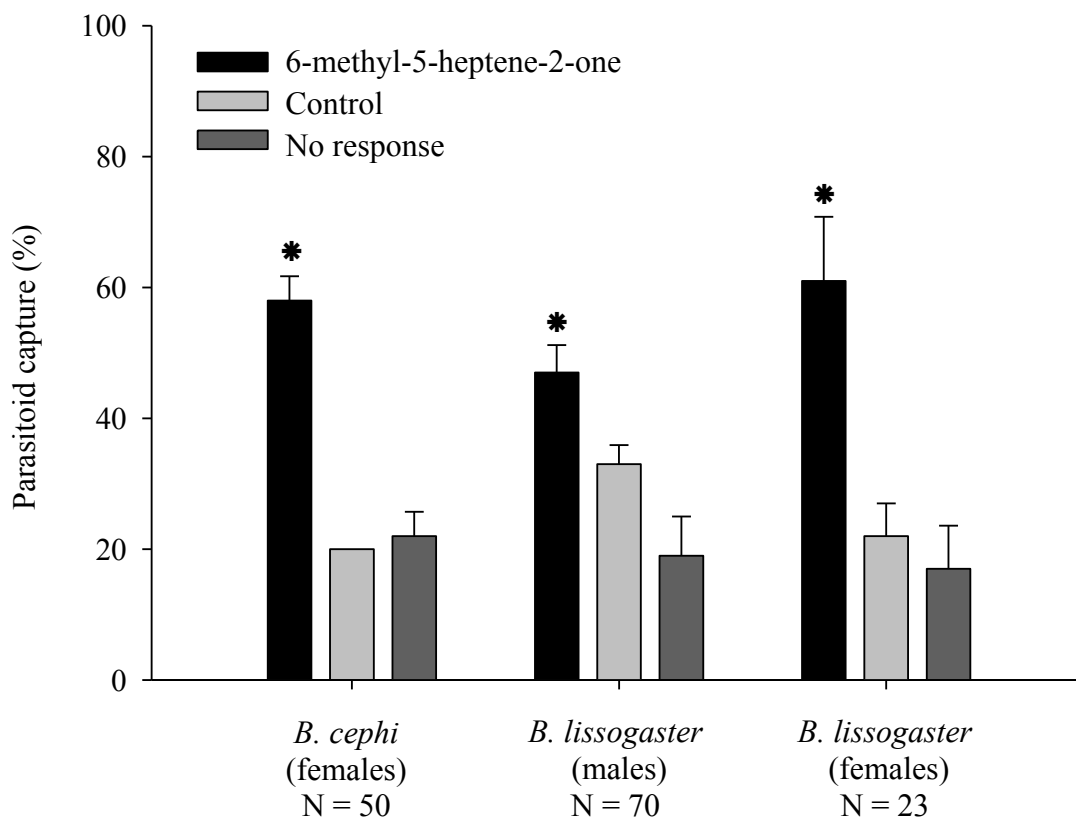


Figure 3.3. Parasitoid behavioral responses to 6-methyl-5-heptene-2-one (35 µg) in a wind tunnel. *: Statistically significant ($\alpha = 0.05$).

Hexahydrofarnesyl acetone was more attractive at lower concentrations of this compound (Figure 3.4). Wind tunnel bioassays using a strong concentration of hexahydrofarnesyl acetone (9 µg) showed no significant response from either *B. cephi* females ($P = 0.44$) or *B. lissogaster* males ($P = 0.17$) (Figure 3.5). There were not enough *B. lissogaster* females to perform statistical analysis. Conversely, the use of a low concentration (0.3 µg) was significantly attractive to both species of parasitoids. *B.*

lissogaster females (64%) showed a strong response to the low concentration of this compound ($P = 0.005$) (Table 3.3). *B. lissogaster* males (60%) and *B. cephi* females (60%) were also significantly attracted to this low concentration ($P < 0.0001$) (Tables 3.1 and 3.2).

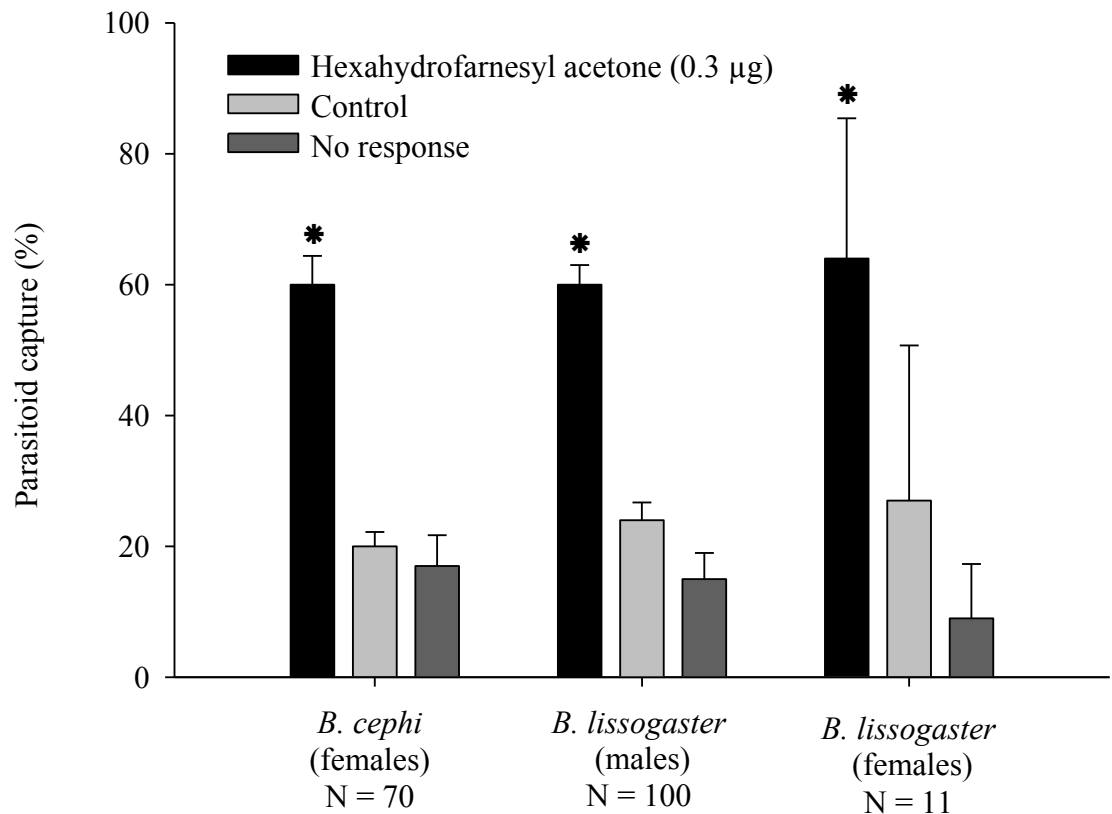


Figure 3.4. Parasitoid behavioral responses to hexahydrofarnesyl acetone (0.3 µg) in a wind tunnel. *: Statistically significant ($\alpha = 0.05$).

There were no significant differences between female and male *B. lissogaster* parasitoid response to hexahydrofarnesyl acetone at low ($P = 0.90$) or high ($P = 0.82$) concentration (Table 3.4).

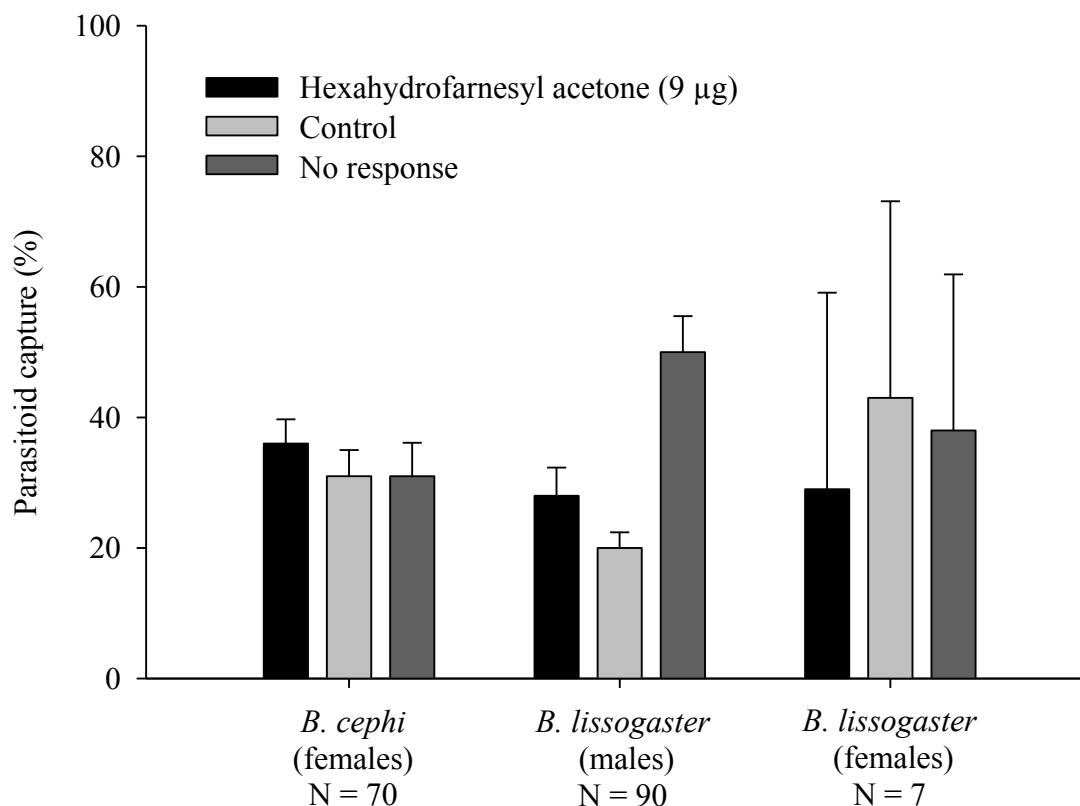


Figure 3.5. Parasitoid behavioral responses to hexahydrofarnesyl acetone (9 µg) in a wind tunnel.

In the electroantennogram tests, other wheat compounds [(*E*)-2-hexenal, benzaldehyde and (*E*)-2-hexenyl acetate], generated some degree of electrophysiological response from the parasitoids. However, in the behavioral trials the response of both parasitoid species to these compounds was not significant. Behavioral tests using (*E*)-2-hexenal produced a 34% response from *B. cephi* females ($P = 0.76$), a 39% response from *B. lissogaster* males ($P = 0.14$), and a 25% response from *B. lissogaster* females. Similar non significant responses were obtained with (*E*)-2-hexenyl acetate and benzaldehyde (Tables 3.1, 3.2, and 3.3).

There was no significant difference in the behavioral responses between *B. lissogaster* males and females for any of the other compounds tested (Table 3.4).

Table 3.1. Behavioral responses of female *B. cephi* parasitoids to isolated, synthetic compounds produced by sawfly infested wheat plants inside a wind tunnel.

Tested Compound	Compound (Parasitoids)		Control (Parasitoids)		<i>t</i> -value	<i>df</i>	<i>P</i> -value
	N	%	N	%			
(<i>Z</i>)-3-hexenyl acetate *	38	63	13	22	-7.90	10	< 0.0001
6-methyl-5-hepten-2-one *	29	58	10	20	-30.96	4	< 0.0001
hexahydrofarnesyl ac. (0.3 µg) *	42	60	14	20	-22.38	12	< 0.0001
hexahydrofarnesyl ac. (9 µg)	25	36	22	31	-0.79	12	0.44
(<i>E</i>)-2-hexenyl acetate	21	35	27	45	-2.06	10	0.07
(<i>Z</i>)-3-hexanol	16	32	14	28	-0.68	8	0.52
(<i>E</i>)-2-hexenal	17	34	16	32	-0.31	8	0.76
Ocimene	17	34	15	30	-0.74	8	0.38

*: Statistically significant ($\alpha = 0.05$); ac.: acetone.

Table 3.2. Behavioral responses of male *B. lissogaster* parasitoids to isolated, synthetic compounds produced by sawfly infested wheat plants inside a wind tunnel.

Tested Compound	Compound (Parasitoids)		Control (Parasitoids)		<i>t</i> -value	<i>df</i>	<i>P</i> -value
	N	%	N	%			
(<i>Z</i>)-3-hexenyl acetate *	51	57	19	21	-8.95	13	< 0.0001
6-methyl-5-hepten-2-one *	33	47	23	33	-17.61	12	< 0.0001
hexahydrofarnesyl ac (0.3 µg) *	60	60	24	24	-8.64	18	< 0.0001
hexahydrofarnesyl ac. (9 µg)	25	28	18	20	-1.44	16	0.17
(<i>E</i>)-2-hexenyl acetate	40	40	42	42	-0.38	18	0.71
(<i>Z</i>)-3-hexanol	17	34	15	30	-0.64	8	0.54
(<i>E</i>)-2-hexenal	35	39	29	32	-1.55	16	0.14
Ocimene	21	35	21	35	-0.02	10	0.99

*: Statistically significant ($\alpha = 0.05$); ac.: acetone.

Table 3.3. Behavioral responses of female *B. lissogaster* parasitoids to isolated, synthetic compounds produced by sawfly infested wheat plants inside a wind tunnel.

Tested Compound	Compound (Parasitoids)		Control (Parasitoids)		<i>t</i> -value	<i>df</i>	<i>P</i> -value
	N	%	N	%			
(<i>Z</i>)-3-hexenyl acetate	8	53	4	27	-2.32	6	0.05
6-methyl-5-hepten-2-one *	14	61	5	22	-8.07	8	< 0.0001
hexahydrofarnesyl ac (0.3 µg) *	7	64	3	27	-4.34	6	0.005
hexahydrofarnesyl ac. (9 µg)	2	29	3	43		--	
(<i>E</i>)-2-hexenyl acetate	6	32	7	37	-1.46	6	0.19
(<i>Z</i>)-3-hexanol	6	32	7	37	-1.19	6	0.28
(<i>E</i>)-2-hexenal	3	25	2	17		--	
Ocimene	9	33	11	41	-1.78	8	0.10

*: Statistically significant ($\alpha = 0.05$); ac.: acetone.

Table 3.4. Comparison of behavioral responses between female and male *B. lissogaster* parasitoids to compounds produced by wheat plants infested by wheat stem sawfly inside a wind tunnel.

Compound	Average responses (%)		<i>t</i>	<i>df</i>	<i>P</i>
	females	males			
(<i>Z</i>)-3-hexenyl acetate *	74	67	1.17	12	0.02
6-methyl-5-hepten-2-one *	74	61	3.22	13	0.007
hexahydrofarnesyl ac. (0.3 µg)	70	71	0.13	3.1	0.90
hexahydrofarnesyl ac. (9 µg)	40	59	-0.25	2.1	0.82
(<i>E</i>)-2-hexenal	60	53	0.29	4.2	0.78
(<i>E</i>)-2-hexenyl acetate	46	49	-0.23	3.2	0.83
(<i>Z</i>)-3-hexanol	46	52	-0.70	4.6	0.52
(<i>E</i>)- β -ocimene	45	52	-0.07	4.7	0.95

*: Statistically significant ($\alpha = 0.05$); ac.: acetone.

Discussion and Conclusions

Earlier electroantennogram tests performed with isolated, synthetic compounds matching those released by wheat plants infested by wheat stem sawflies revealed that (Z)-3-hexenyl acetate, 6-methyl-5-heptene-2-one, and hexahydrofarnesyl acetone generated a significant and consistent electrophysiological response from *B. cephi* and *B. lissogaster* (Chapter 2, “Electroantennogram responses of parasitoids *Bracon cephi* and *Bracon lissogaster* to synthetic wheat volatiles matching those released by wheat plants infested with wheat stem sawfly”).

(Z)-3-hexenyl acetate, 6-methyl-5-heptene-2-one, and hexahydrofarnesyl acetone also generated a consistent and significant attractive response from *B. cephi* and *B. lissogaster*. (Z)-3-hexenyl acetate has been found to be induced in corn (*Zea mays* L.) seedlings by caterpillar oral secretions and to be attractive to generalist and specialized parasitic wasps (Turlings et al. 1993). It was also found to attract *Trichogramma chilonis* (Ishii) and *Cotesia plutellae* (Kurdjumov), parasitoids of the diamondback moth, *Plutella xylostella* L. (Reddy et al. 2002). Feeding by the pea aphid, *Acyrtosiphon pisum* (Harris) on broad bean, *Vicia faba* L. increased the production of 6-methyl-5-heptene-2-one, which was found to be very attractive for the aphid parasitoid *Aphidius ervi* Haliday (Du et al. 1998). These results suggest the possibility of these compounds working as chemical cues indicating infested stems for these parasitoid species. Further studies are required in order to determine the most effective amounts, and the possible effects of the application of the synthetic compound on natural occurring rates of parasitism.

There appears to be a significantly higher percentage of females (compared to males) that are attracted to (Z)-3-hexenyl acetate and 6-methyl-5-heptene-2-one. Considering that females are the ones that oviposit on the wheat stem sawfly hosts inside the stem, there is some likelihood of a stronger response from the females to these specific compounds.

Hexahydrofarnesyl acetone, a ripening compound (Miyazawa et al. 2008), is an important compound to take into consideration given its unusual activity (Chapter 2, “Electroantennogram responses of parasitoids *Bracon cephi* and *Bracon lissogaster* to synthetic wheat volatiles matching those released by wheat plants infested with wheat stem sawfly”). This compound is not reported to have behavioral activity in many systems although it is known to attract Euglossini bees (Eltz and Lunau 2005). The consistent and significant electrophysiological responses, the attractiveness of this compound to the parasitoids at low concentrations, and the reduction in the production of this compound by infested wheat plants to low airborne amounts during the parasitoid flight period (Chapter 4, “Quantification and spatial distribution of behaviorally active volatiles from infested and uninfested plants using an open volatile collection system in the greenhouse” and Chapter 5, “Influence of wheat stem sawfly infestation level on localized variability of behaviorally active compounds in wheat fields”), makes it a very promising compound to further test as a *B. cephi* and *B. lissogaster* attractant in sawfly infested fields. There are cases of specific parasitoids responding only to very low concentrations of a specific compound. Electrophysiological responses from the antenna of *Cotesia glomerata* L., a generalist parasitoid, and *Cotesia rubecula* Marshall, a

specialist on *Pieris rapae* L., were both positive to a specific group of compounds produced by Brussels sprouts (*Brassica oleracea* L.). However, there was one compound produced at very low concentrations to which only the specialist parasitoid responded (Smid et al. 2002). In relation to this behavior Geervliet et al. (2000) mentioned, “An increased olfactory sensitivity would be adaptive for *C. rubecula*, since it is a specialist on a solitary host and its feeding will induce lower amounts of plant volatiles than the gregariously feeding *P. brassicae*, which is the preferred host of the generalist *C. glomerata*”.

Even though (*E*)-2-hexenal, (*E*)-2-hexenyl acetate, and benzaldehyde are compounds with activity on the parasitoid antennae at different concentrations (Chapter 2, “Electroantennogram responses of parasitoids *Bracon cephi* and *Bracon lissogaster* to synthetic wheat volatiles matching those released by wheat plants infested with wheat stem sawfly”), these behavioral tests determined that at the tested concentrations these compounds are not likely to be used as chemical cues in host seeking. Other behavioral tests using a range of concentrations of these compounds are required to rule out if these compounds play any role in the host seeking behavior of these braconid parasitoids, but these are beyond the scope of the current research.

We have determined that there is the possibility that variation in the amounts of compounds present in wheat plants that are either uninfested or infested by wheat stem sawfly could be used as possible cues in host seeking by these braconid parasitoids. This possibility is based on the analysis of the electrophysiological and behavioral responses of the parasitoids to a suite of compounds previously identified as varying as a result of

infestation in closed volatile collections (Peck 2004) and also those with activity to adults of the host (Piesik et al. 2008). Piesik et al. (2008) presents solid evidence of four wheat volatile compounds attracting adult female sawflies. Behavioral trials using an olfactometer system revealed that (*Z*)-3-hexenyl acetate, 6-methyl-5-heptene-2-one, (*Z*)-3-hexenol, and (*E*)- β -ocimene, generated behavioral activity on adult female sawflies at different concentrations. (*Z*)-3-hexenyl acetate showed to attract female sawflies at concentrations of 60, 120, and 180 ng hr⁻¹, (*Z*)-3-hexenol was attractive to females at 6 ng hr⁻¹, and (*E*)- β -ocimene at 0.96 ng hr⁻¹. Conversely, 6-methyl-5-heptene-2-one was found to repel female sawflies at 42 ng hr⁻¹. Males were not found to be attracted to any of the tested wheat compounds. Because adult sawflies do not feed, searching for a host is probably not a priority for the males. They are probably more adapted to pheromones used in mating (Bartelt et al. 2002, Cossé et al. 2002), so receptors might be more specialized for pheromone detection. The results obtained by Piesik et al. (2008) provide encouragement in finding a similar system between the braconid parasitoids and the wheat plants.

This wheat-sawfly-parasitoid system would have to work based on relative differences in the amount of compounds in a characteristic blend, with associative learning by experience (Turlings et al. 1989, Lewis et al. 1991, McCall et al. 1993, Röse et al. 1997, Fukushima et al. 2002) for foraging, successful females in the second generation. In addition, it has also been demonstrated that this experience can often come from contact with the substrate the parasitoid has been associated with (Ngi-Song and Overholt 1997). Therefore, this bivoltine species might readily experience cues

associated with infested stems during the process of chewing an exit hole in the side of the stem as it emerges to begin the second generation (Holmes and Farstad 1956, Somsen and Luginbill 1956). However, this experience would not be available to overwintering parasitoids that emerge from the distinctly different overwintered postharvest residue the following spring. Furthermore, we see that positive behavioral responses suggest that conclusions about the failure to induce a unique signal being a limitation in parasitoid host seeking of stem dwelling insect larvae (Tooker and De Moraes 2007) may be challenged. In particular, finding a wheat stem containing a cryptic larva of the wheat stem sawfly necessitates some form of discrimination that can be made during host seeking within the crop canopy. Therefore, the following chapters will address possible discriminatory differences in volatile emissions that may be utilized during host seeking by these parasitoids.

Further studies need to be undertaken to assess the response of *B. lissogaster* female parasitoids to hexahydrofarnesyl acetone (9 µg) and (*E*)-2-hexenal, which was not possible because of the inavailability of sufficient numbers of parasitoids.

References

- Agelopoulos, N., M. A Birkett, A. J. Hick, A. M. Hooper, J. A. Pickett, E. M. Pow, L. E. Smart, D. W. M. Smiley, L. J. Wadhams, and C. M. Woodcock. 1999. Exploiting semiochemicals in insect control. *Pestic. Sci* 55:225-235.
- Ainslie, C. N. 1920. The western grass-stem sawfly. U.S. Department of Agriculture Bulletin. p 841.
- Agbogba, B. C. and W. Powell. 2007. Effect of the presence of a nonhost herbivore on the response of the aphid parasitoid *Diaeretiella rapae* to host-infested cabbage plants. *J. Chem. Ecol.* 33: 2229-2235.
- Bartelt, R. J, A. A. Cossé, R. J. Petroski, and D. K. Weaver. 2002. Cuticular hydrocarbons and novel alkenediol diacetates from wheat stem sawfly (*Cephus cinctus*): natural oxidation to pheromone components. *J. Chem. Ecol.* 28:385-405.
- Bernasconi, M. L., T. C. J. Turlings, L. Ambrosetti, P. Bassetti, and S. Dorn. 1998. Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, *Rhopalosiphum maidis*. *Entomol. Exp. Appl.* 87: 133-142.
- Bruce, T. J. A., L. J. Wadhams, and C. M. Woodcock. 2005. Insect host location: a volatile situation. *Trends Plant Sci.* 10: 269-274.
- Callenbach, J. A. and M. P. Hansmeier. 1945. Wheat stem sawfly control. Montana Extension Service in Agriculture and Home Economics, Circular No. 164.
- Cortesero, A. M., J. O. Stapel, and W. J. Lewis. 2000. Understanding and manipulating plant attributes to enhance biological control. *Biol. Control* 17: 34-49.
- 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. *J. Chem. Ecol.* 28: 407-423.
- D'Alessandro, M. and T. C. J. Turlings. 2005. *In situ* modification of herbivore-induced plant odors: a novel approach to study the attractiveness of volatile organic compounds to parasitic wasps. *Chem. Senses* 30: 739-753.
- De Moraes C. M., W. J. Lewis, P. W. Paré, H. T. Alborn, and J. H. Tumlinson. 1998. Herbivore-infested plants selectively attract parasitoids. *Nature* 393: 570-573.
- Degenhardt, J. 2009. Indirect defense response to herbivory in grasses. *Plant Physiol.* 149: 96-102.

- Dorn, S. and A. Hern. 1999. Herbivore-induced volatiles in the apple orchard ecosystem. IOBC wprs Bulletin 22: 1-5.
- Du, Y., G. M. Poppy, W. Powell, J. A. Pickett, L. J. Wadhams, and C. M. Woodcock. 1998. Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. J. Chem. Ecol. 24: 1355-1368.
- Dudareva, N., E. Pichersky, and J. Gershenzon. 2004. Biochemistry of plant volatiles. Plant Physiol. 135: 1893-1902.
- Eltz, T. and K. Lunau. 2005. Antennal response to fragrance compounds in male orchid bees. Chemoecology 15:135-138.
- Elzen, G. W., H. J. Williams, and S. B. Vinson. 1986. Wind tunnel flight responses by hymenopterous parasitoid *Campoletis sonorensis* to cotton cultivars and lines. Entomol. Exp. Appl. 42: 285-289.
- 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.
- Fatouros, N. E., J. J. A. Van Loon, K. A. Hordijk, H. M. Smid, and M. Dicke. 2005. Herbivore-induced plant volatiles mediate in-flight host discrimination by parasitoids. J. Chem. Ecol. 31: 2033-2047.
- Foster, S. P. and M. O. Harris. 1997. Behavioral manipulation methods for insect pest-management. Annu. Rev. Entomol. 42: 123-146.
- Fukushima, J., Y. Kainoh, H. Honda, and J. Takabayashi. 2002. Learning of herbivore-induced and nonspecific plant volatiles by a parasitoid, *Cotesia kariyai*. J. Chem. Ecol. 28: 579-586.
- Geervliet, J. B. F., M. S. W. Verde, J. Schaub, H. Snellen, M. Dicke, and L. E. M. Vet. 2000. Coexistence and niche segregation by field populations of the parasitoids *Cotesia glomerata* and *C. rubecula* in the Netherlands: predicting field performance from laboratory data. Oecologia 124:55-63.
- Goff, S. A. and J. H. Klee. 2006. Plant volatile compounds: sensory cues for health and nutritional value? Science 311: 815-819.
- Hartel, K. D., B. A. Frederick, and T. G. Shanower. 2003. Isolation and characterization of microsatellite loci in wheat stem sawfly *Cephus cinctus* and cross-species amplification in related species. Molec. Ecol. Notes. 3 p.

- Heil, M., U. Lion, and W. Boland. 2008. Defense-inducing volatiles: In search of the active motif. *J. Chem. Ecol.* 34:601-604.
- Holmes, N. D. 1954. Food relations of the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae). *Can. Entomol.* 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). *Can. J. Agric. Sci.* 36: 196-202.
- 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. *Can. Entomol.* 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. *Can. Entomol.* 109: 1591-1598.
- Holmes, N. D. 1978. The wheat stem sawfly. *Proc. 26th Annu. Mtg. Entomol. Soc. Alberta.* pp. 2-13.
- Holopainen, J. K. 2004. Multiple functions of inducible plant volatiles. *Trends Plant Sci.* 9: 529-533.
- Ichiki, R. T., Y. Kainoh, S. Kugimiya, J. Takabayashi, and S. Nakamura. 2008. Attraction to herbivore-induced plant volatiles by the host-foraging parasitoid fly *Exorista japonica*. *J. Chem. Ecol.* 34:614-621.
- James, D. G. 2003. Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: methyl salicylate and the green lacewing, *Chrysopa nigricornis*. *J. Chem. Ecol.* 29: 1601-1609.
- Kessler, A., and T. Baldwin. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291: 2141-2144.
- Lewis, W. J., J. H. Tumlinson, and S. Krasnoff. 1991. Chemically mediated associative learning: an important function in the foraging behavior of *Microplitis croceipes* (Cresson). *J. Chem. Ecol.* 17: 1309-1325.
- Lo Pinto, M., E. Wajnberg, S. Colazza, C. Curty, and X. Fauvergue. 2003. Olfactory response of two aphid parasitoids, *Lysiphlebus testaceipes* and *Aphidius colemani*, to aphid-infested plants from a distance. *Entomol. Exp. Appl.* 110: 159-164.

- Mattiacci, L., M. Dicke, and M. A. Posthumus. 1995. β -Glucosidase: An elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. *Proc. Natl. Acad. Sci. USA* 92: 2036-2040.
- McCall, P. J., T. C. Turlings, W. J. Lewis, and J. H. Tumlinson. 1993. Role of host plant volatiles in host location by the specialist parasitoid *Microplitis croceipes* Cresson (Braconidae: Hymenoptera). *J. Insect Behav.* 6: 625-639.
- Miyazawa, M., S. Nagai, and T. Oshima. 2008. Volatile components of the straw of *Oryza sativa* L. *J. Oleo Sci.* 57: 139-144.
- 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. *J. Econ. Entomol.* 87: 1373-1376.
- Ngi-Song, A. J., and W. A. Overholt. 1997. Host location and acceptance by *Cotesia flavipes* Cameron and *C. sesamiae* (Cameron) (Hymenoptera: Braconidae), parasitoids of African gramineous stemborers: role of frass and other host cues. *Biol. Control* 9: 136-142.
- Ode, P. J. 2006. Plant chemistry and natural enemy fitness: Effects on herbivore and natural enemy interactions. *Annu. Rev. Entomol.* 51:163-85.
- Paré, P. W. and J. H. Tumlinson. 1999. Plant volatiles as a defense against insect herbivores. *Plant Physiol.* 121: 325-331.
- Peck, G. E. 2004. Potential semiochemicals of wheat (*Triticum aestivum* L.) induced by oviposition and feeding of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). [Master's Thesis]. Entomology, Montana State University, Bozeman, Montana. 65 p.
- Peñuelas, J. and J. Llusà. 2004. Plant VOC emissions: making use of the unavoidable. *Trends Ecol. Evol.* 19: 402-404.
- Pettersson, E. M. and W. Boland. 2003. Potential parasitoid attractants, volatile composition throughout a bark beetle attack. *Chemoecology* 13: 27-37.
- Pichersky, E. and D. R. Gang. 2000. Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. *Trends Plant Sci.* 5: 439-445.
- Pichersky, E., J. P. Noel, N. Dudareva. 2006. Biosynthesis of plant volatiles: Nature's diversity and ingenuity. *Science* 311: 808-811.

- Pickett, J. A., L. J. Wadhams, and C. M. Woodcock. 1997. Developing sustainable pest control from chemical ecology. *Agric. Ecosys. Environ.* 64: 149-156.
- Piesik, D., D. K. Weaver, J. B. Runyon, M. Buteler, G. E. Peck, and W. L. Morrill. 2008. Behavioral responses of wheat stem sawflies to wheat volatiles. *Agric. For. Entomol.* 10: 245-253.
- Reddy, G. V. P., J. K. Holopainen, and A. Guerrero. 2002. Olfactory responses of *Plutella xylostella* natural enemies to host pheromone, larval frass, and green leaf cabbage volatiles. *J. Chem. Ecol.* 28: 131-143.
- Röse, U. S. R., H. T. Alborn, G. Makranczy, W. J. Lewis, and J. H. Tumlinson. 1997. Host recognition by the specialist endoparasitoid *Microplitis croceipes* (Hymenoptera: Braconidae): role of host- and plant-related volatiles. *J. Insect Behav.* 10: 313-329.
- Runyon, J. B., Morrill, W. L., Weaver, D. K., Miller, P. R. 2002. Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) by *Bracon cephi* and *B. lissogaster* (Hymenoptera: Braconidae) in wheat welds bordering tilled and untilled fallow in Montana. *J. Econ. Entomol.* 95: 1130-1134.
- Rott, A. S., J. Häckermann, N. Brand, A. Vallat, and S. Dorn. 2005. Parasitoid exploitation of the seasonal variation in host plant volatile emission for herbivore location. *Entomol. Exp. Appl.* 115: 199-205.
- Sabelis, M. W., A. Janssen, and M. R. Kant. 2001. Enhanced: the enemy of my enemy is my ally. *Science* 291: 2104-2105.
- 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 Entomol. Soc. Annu. Rpt.* 75: 10-15.
- Smid, H. M., J. J. A. van Loon, M. A. Posthumus and L. E. M. Vet. 2002. GC-EAG-analysis of volatiles from Brussels sprouts plants damaged by two species of *Pieris* caterpillars: olfactory receptive range of a specialist and a generalist parasitoid wasp species. *Chemoecology* 12:169-176.
- Somsen, H. W. and P. Luginbill. 1956. *Bracon lissogaster* Muesbeck, a parasite of the wheat stem sawfly. *USDA, Tech. Bull. No.* 1153.
- Tooker, J. F. and C. M. De Moraes. 2007. Feeding by Hessian fly [*Mayetiola destructor* (Say)] larvae does not induce plant indirect defenses. *Ecol. Entomol.* 32: 153-161.

- Tumlinson, J. H., W. J. Lewis, and E. M. Louise. 1993. How parasitic wasps find their hosts. *Sci. Am.* 264: 100-106.
- Turlings, T. C., J. H. Tumlinson, and W. J. Lewis, and L. E. M. Vet. 1989. Beneficial Arthropod behavior mediated by airborne semiochemicals. VIII. Learning of host-related odors induced by a brief contact experience with host by-products in *Cotesia marginiventris* (Cresson), a generalist larval parasitoid. *J. Insect Behav.* 2: 217-25.
- Turlings, T. C., J. H. Tumlinson, and W. J. Lewis. 1990. Exploitation of herbivore induced plant odors by host-seeking parasitic wasps. *Science* 250: 1251-1253.
- Turlings, T. C. J., P. J. McCall, H. T. Alborn, and J. H. Tumlinson. 1993. An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *J. Chem. Ecol.* 19: 411-425.
- Turlings, T. C. J., M. Bernasconi, R. Bertossa, F. Bigler, G. Caloz, and S. Dorn. 1998. The induction of volatile emissions in maize by three herbivore species with different feeding habits: Possible consequences for their natural enemies. *Biol. Control* 11: 122-129.
- Vinson, S. B. 1976. Host selection by insect parasitoids. *Annu. Rev. Entomol.* 21:109-133.
- Weaver, D. K., S. E. Sing, J. B. Runyon, and W. L. Morrill. 2004. Potential impact of cultural practices on wheat stem sawfly (Hymenoptera: Cephidae) and associated parasitoids. *J. Agric. Urban. Entomol.* 21: 271-287.
- 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. *Biol. Control* 34: 1-11.
- Weiss, M. J. and W. L. Morrill. 1992. Wheat stem sawfly (Hymenoptera: Cephidae) revisited. *Am. Entomol.* 38: 241-245.
- Williams III, L., C. Rodriguez-Saona, S. C. Castle, and S. Zhu. 2008. EAG-active herbivore-induced plant volatiles modify behavioral responses and host attack by an egg parasitoid. *J. Chem. Ecol.* 34:1190-1201.

CHAPTER 4

QUANTIFICATION AND SPATIAL DISTRIBUTION OF BEHAVIORALLY
ACTIVE VOLATILES FROM SAWFLY INFESTED AND UNINFESTED
WHEAT PLANTS USING AN OPEN VOLATILE COLLECTION
SYSTEM IN THE GREENHOUSE

Introduction

Plants are known to produce volatile organic compounds which are typically associated with the fragrance of the plant; although they may often be present in small quantities that do not contribute to human perception of scent (Lerdau et al. 1997). These compounds, typically hydrocarbons, are produced in varying amounts in plants by external and vascular tissues of different organs (flowers, leaves, fruits, roots and stems) (Knudsen et al. 1993, Hildebrand et al. 1993, Konstantopoulou et al. 2004, Peck 2004, Tholl et al. 2006), and have been divided in two groups: green leaf volatiles, which are volatile compounds produced by most plants in varying amounts, and a group of more unique compounds that are produced by specific groups of plants and often only under certain conditions (Hansson et al. 1999).

It has been determined that plants release volatile chemicals that work as airborne cues in the attraction of specific pests (Dudareva et al. 2004, Peck 2004, Bruce et al. 2005, Buteler 2008). In a number of cases, attractants have been used as chemical lures in pest control (Jacobson and Beroza 1963, Foster and Harris 1997, Epsky and Heath 1998). Some of these compounds are also known to attract biological control agents and

play a key role in their ability to control the pest (Lewis et al. 1975, Pickett et al. 1997, Fatouros et al. 2008). However, in a natural setting many factors need to be considered and determined to effectively measure attractive compounds and determine how they are distributed. Once released from the plant, resistance to ultraviolet light, temperature, humidity, airflow will influence the potential attractiveness, as will the chemical properties such as molecular weight, volatility, and reactivity to oxygen. These factors, among others, control the pattern of dispersion of volatile molecules into the airspace surrounding the plant (Takabayashi et al. 1994, Lerda et al. 1997, Schoonhoven et al. 2006).

There are other factors that can influence the production and distribution of these compounds. Plant density, plant developmental stage, photoperiod, and the infestation level by a pest will influence the concentration and dispersion of these compounds (Agelopoulos et al. 2000, Peck 2004). The vapor pressure of these volatile compounds is typically greater than for air, and certainly will vary among compounds, which will influence their relative dissipation.

This study is centered on determining if volatile compound amounts from wheat stem sawfly infested wheat (*Triticum aestivum* L.) plants are different from those from uninfested at two distances from the stem, and at several heights along the stem. Another experiment will analyze the same factors, but with the infested plant growing among uninfested wheat plants. The wheat stem sawfly is one of the main insect pests of wheat in the Northern Great Plains of North America (Weiss and Morrill 1992, Morrill et al. 2001, Beres et al. 2007). This pest causes damage as a stem boring larva that lives inside

the wheat stem feeding on the parenchymous and vascular tissues of the stems (Holmes 1977). Because of the cryptic nature of this pest, it has been very difficult to control.

Within the wheat stem sawfly system there is a tritrophic system that has been found to show promise in effective suppression of this pest (Weaver et al. 2005, Buteler et al. 2008). Chemical interactions that have been found between wheat plants, the wheat stem sawfly, and two braconid parasitoid species (*Bracon cephi* (Gahan) and *Bracon lissogaster* Muesebeck) (Peck 2004, Piesik et al. 2008, Chapter 2: “Electroantennogram responses of parasitoids *Bracon cephi* and *Bracon lissogaster* to synthetic wheat volatiles matching those released by wheat plants infested with wheat stem sawfly”, Chapter 3: “Behavioral responses of the parasitoids *Bracon cephi* and *Bracon lissogaster* to isolated synthetic volatiles matching those released by wheat plants that are infested by the wheat stem sawfly”), suggest the possibility of using chemical ecology as a tool in developing more effective methods to manage this pest using these braconid parasitoids.

Host location by the natural enemies of herbivores relies mainly on chemical stimuli, be it stimuli from the host habitat or food plant, or stimuli associated with the presence of the host itself (Godfray and Waage, 1988). Numerous studies on tritrophic systems involving a natural enemy, such as a parasitoid, the herbivorous insect, and a plant have shown that parasitoids use a variety of cues in deciding where to search and for how long (Vet et al. 1995, Vinson 1998), and that chemical information from the plant is generally considered to play a pivotal role in the location, evaluation, and parasitism of a host by its parasitoid (Dicke and van Loon 2000, Mattiacci et al. 2001). Therefore, temporal changes in plant-derived chemical stimuli will affect interactions with herbivores as well

as natural enemies. For example, the volatile blend emitted from apple (*Malus domestica* Borkhausen) fruit *in situ* changes drastically both in quantity and chemical composition as the season progresses (Bengtsson et al. 2001, Hern and Dorn 2003).

In these experiments we will analyze the cumulative effect of different factors (plant height, distance from plant, sawfly infestation, and separately, the presence of competing uninfested wheat plants), on potential differences in the amount initially released from infested plants. This will be accomplished by collecting volatiles from the open airspace, which is distinct from how this research is typically conducted (Peck 2004, Tholl et al. 2006).

Preliminary observations from pilot experiments performed by collecting volatile compounds from sawfly infested wheat plants at different plant heights suggested that there are patterns of volatile compound dissipation throughout the canopy of the wheat plants. Due to limited air movement within a dense canopy and the fact that these compounds are heavier than air, these compounds may accumulate at the base of the plants.

Collecting this data on volatile dispersal patterns will allow us to study how parasitoids could orient toward wheat stems containing hosts by being able to distinguish the differing amounts due to infestation at different locations, which will then be further addressed by subsequently measuring odor patterns in a canopy of uninfested plants. Characterizing compounds that are detectable in emanations from the plant allow for the possibility of using one or several of these compounds in the process of better controlling the wheat stem sawfly with biological control agents (Peck 2004, Buteler 2008). This

study will attempt to address paradigms that have been established regarding the limitations of plant volatiles in host seeking of stem borers in grasses (Tooker and De Moraes 2007).

Objectives

Main Objective

Clarify preliminary observations that volatile compounds from infested plants may accumulate at the base of the plants and determine if there is an effect of height on collection of behaviorally-active volatiles that is distinct from that associated with uninfested plants. This would also encompass different rates of volatile production by different parts of both infested and uninfested plants.

Specific Objectives

1. Analyze the effect of wheat stem sawfly infestation on the amount of behaviorally-active volatile compounds collected from wheat plants.
2. Establish the effect that uninfested wheat plants growing with a sawfly infested plant have on the amount of behaviorally-active volatile compounds distributed within the canopy compared to the amount collected from a canopy consisting only of uninfested plants.
3. Determine if there is a significant difference in the amounts of behaviorally-active plant volatiles collected at different heights adjacent to the stem and at 10 cm away from the stem for infested and uninfested plants.

Test Hypotheses

Test Hypothesis 1: Sawfly infestation of the plants will have a significant effect on the amount of behaviorally-active volatiles collected.

Test Hypothesis 2: The presence of uninfested wheat plants with a sawfly infested plant will have a significant effect on the collection and distribution of behaviorally-active volatile compounds.

Test Hypothesis 3: There will be a significant decrease in the overall amount and ratio of behaviorally-active compounds collected from the infested wheat plants relative to the uninfested plants as the collection height and distance from the stem increases.

Materials and Methods

Plant Material

Wheat of the variety ‘McNeal’ was planted at two different densities. One group consisted of twenty five round (23 cm height, 23 cm diameter) plastic pots (Gage Durapots™, Lake Oswego, Oregon), each one planted with thirteen wheat plants. The other group consisted of twenty five square (14 cm height, 13 cm side) plastic pots planted with three plants. Two sets of each plant group were planted 14 days apart under the same conditions. The soil used was a 50/50 mix of PGC Soil Mix (equal parts of Bozeman silt loam soil / washed concrete sand / Canadian sphagnum peat moss.

AquaGro 2000 G wetting agent (aerated steam pasteurized at 80 °C for 45; pH ~ 6-7) is blended in at one pound per cubic yard of soil mix, and Sunshine Mix # 1 (Canadian sphagnum peat moss, perlite, vermiculite, starter nutrient charge, wetting agent, and

dolomitic lime; pH ~ 5.5- 6) (Sun Gro Horticulture Inc., Bellevue, Washington). All the pots from both groups were filled with equal amounts of the soil mix and were kept in a clean, insect-free walk-in Conviron growth chamber under controlled temperature (Day: $22^{\circ}\text{C} \pm 1.5^{\circ}\text{C}$; Night: $18^{\circ}\text{C} \pm 1.5^{\circ}\text{C}$), and day length (15L: 9D) conditions. The plants were watered at least two times a week and fertilized (Peters® 20-20-20 general mix: 7.9 g/l water) twice a week at 500 ml per pot.

Two weeks after planting, the plants in each pot were thinned out to a final density of one plant per pot (isolated plant group) and ten plants per pot (low infestation density group). The plants were grown in an insect-free chamber under the same controlled conditions until they reached a developmental stage between Zadoks 32 (2nd node detectable) and Zadoks 34 (4th node detectable) (Zadoks et al. 1974). After reaching this stage the plants were moved into a greenhouse with controlled temperature (Day: $22^{\circ}\text{C} \pm 1.5^{\circ}\text{C}$ [lights on]; Night: $20^{\circ}\text{C} \pm 1.5^{\circ}\text{C}$ [lights off]), and day length (15L: 9D) conditions. After two or three days of adaptation to the new conditions in the greenhouse, the plants were infested with adult wheat stem sawflies.

Sawfly Infestation

The process of infestation consisted of placing a transparent plastic cylinder (60-65 cm long and 3.8 cm diameter) over the main stem of the plant to be infested and introducing one male and four female sawflies. Each cage had three round holes (3.8 cm diameter) covered with white fine cloth-mesh distributed along the cylinder for air circulation and it had an additional 1.1 cm diameter hole that was used to introduce the sawflies and was then sealed using a rubber cork. Soil was mounded around the bottom

of each cylinder to prevent the sawflies from escaping. Cylinders were also placed over the control plants but no sawflies were added.

From both groups, twenty pots were infested with sawflies and the other five were used as controls. The single plant growing in isolation and one plant from the group of ten plants were infested. Thus, the single infested plant represented the greatest likelihood of detecting infestation, while the single infested plant among ten represented a lower likelihood due to the lower infestation density.

The sawflies oviposited for five days after which they and the cylindrical cages were removed. During the oviposition period, the plants were watered and fertilized as previously described.

Volatile Collection

Volatile collections were initiated when the plants were in anthesis (Zadoks 60), allowing longer development of the sawfly larvae, which should produce greater damage to the plant (Peck 2004) and therefore cause the emission of greater amounts of volatiles that may be influenced by herbivory. Also, the parasitoids require larger larvae as hosts, and in the field parasitized larvae first appear during the later developmental stages of the wheat plants. Volatile collections were performed once a week over a four week period.

For this experiment three collection heights and two collection distances relative to the infested plants were used. The collection heights were set along the side of the plant and distributed uniformly starting at the base of the plant, at $\frac{1}{3}$, and at $\frac{2}{3}$ of the plant's height. The collection distances were adjacent to and 10 cm from the plant stem.

Volatile collections were performed for all three heights, but at only one distance at a

time to prevent altering the accumulation of dissipated volatiles for a particular pot. This was accomplished for the volatile collections adjacent to one half of the plants by attaching three collection filters to a bamboo stick at the three previously mentioned plant heights and inserting the stick with the filters adjacent to the plant stem. For the volatile collections at 10 cm from the stem, the three collection filters were attached to a metal stand at the three previously mentioned plant heights and the stand with the filters was placed 10 cm away from the other plant stems. Infested and uninfested plants were collected identically and at the same time.

Within the low infestation density group of plants, in order to detect any possible effect in the dispersion of the volatiles caused by the presence of uninfested plants, the collection filters were placed at two different positions when collecting at 10 cm from the stem: 1) Outside of the pot (on a metal stand beside the pot at 10 cm from the infested plant stem) with no plants surrounding the metal stand with the filters and with no uninfested plants in between the infested plant and the stand; 2) Inside the pot (on a bamboo stick inserted into the soil at 10 cm from the infested plant) with uninfested plants standing between the infested plant and the bamboo stick with the collection filters, and around the bamboo stick as well. The idea of implementing this variation in the placement of the filters was to determine if the difference in the amount of some compounds produced by infested relative to uninfested plants would not be detectable when collecting at a greater distance. Conversely, we also wanted to know if there might be a difference in accumulation of some of the heavier volatile compounds (higher

molecular weight than air) at the soil surface of the pot when collecting from inside the pot, when comparing infested and uninfested plants.

The volatile collections were performed with a portable volatile collection system. This system is a modification of the system originally described by Heath and Manukian (1992) and collects air in an open system in contrast with the closed system, as was designed by Heath and Manukian (1992).

This volatile collection system is powered by a 1 kW/m² - 1.5A portable solar battery charger panel (Model FLX-05, Uni-Solar, Auburn Hills, Missouri) regulated by a 12V / 4.5A solar controller (SunGuard, Morningstar Corporation). The panel collects the solar energy and transforms it into electrical power that is regulated by the solar controller, preventing battery overcharge. The battery is a 12V / 3.4A sealed rechargeable battery (Model PS-1230, Power-Sonic Corporation), that powers a 12V motor/diaphragm vacuum pump system (Model 5002-0408 DC-PM, 3000 rpm, Rietschle Thomas) controlled by an on/off electrical light switch. This part of the system produces the vacuum required to draw the air from the collecting point. The vacuum is established using Tygon® tubing connections to a pair of flowmeters (Series MR 3000, Model 3A13, Key Instruments) that regulate the airflow volume. The last part of the system consists of two 1.5 m Tygon® tubes (0.95 cm OD x 0.64 cm ID), each one extending from the flowmeter to a volatile collection trap (6.35 mm-OD, 76 mm long glass tube; Analytical Research Systems, Inc.) containing 30 mg of Super-Q (Alltech Associates, Inc., Deerfield Illinois) adsorbent. The volatiles collected were retained inside this adsorbent trap and eluted immediately after the collection terminated. The system described above is

assembled inside a plastic container (25 x 25 x 20 cm), containing a smaller metal box (15 x 15 x 7 cm) connected to it by plastic tubing. The smaller box has two mounted flowmeters that control airflow; these are connected to the collecting traps by the Tygon[®] tubing. The box is also used to store all the Tygon[®] tubing in the system (Figure 4.1).

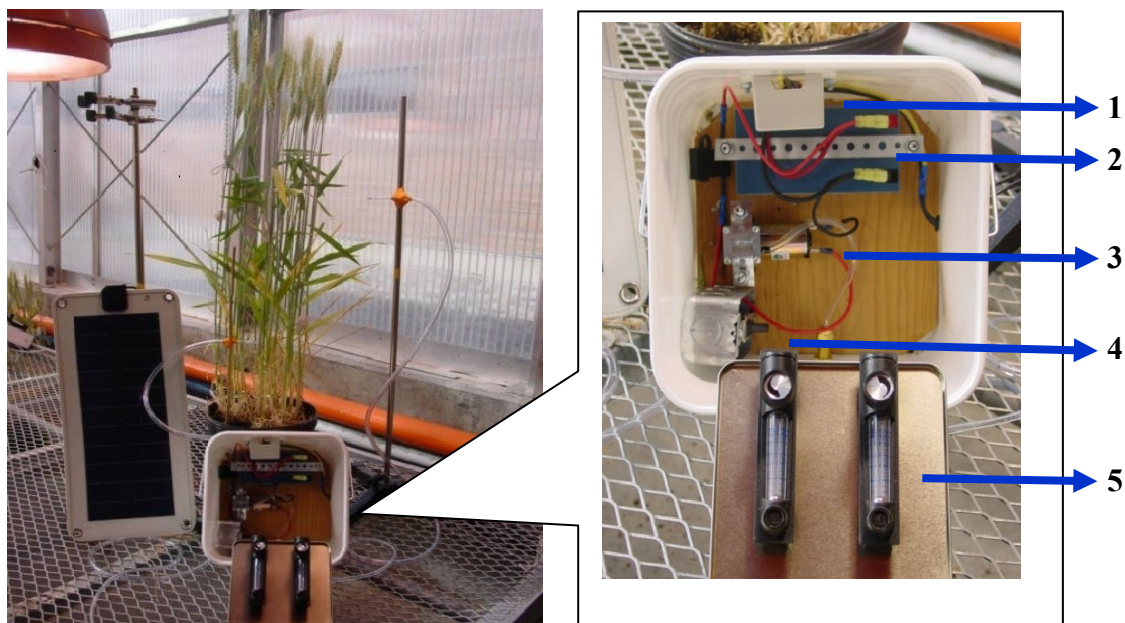


Figure 4.1. Portable volatile collecting apparatus (PVCS).
Components and experimental layout of the portable volatile collection system:

1. Solar controller.
2. Rechargeable battery (12 volt).
3. Diaphragm vacuum pump.
4. Electric on/off wall switch.
5. Flowmeter (1.2 l min⁻¹).

Before starting the collection, all batteries were charged to an adequate voltage (at least 11.5 volts) to ensure the system would operate for the entire collection interval if it is cloudy. The collection rate used was 0.35 l min^{-1} .

The process began with the measurement of plant height and the calculation of the specific heights for collection adjacent to each plant. The volatile collection filters (or traps) were secured to a metal stand or to a bamboo stick inserted in the soil with the filter traps held with wire twist ties (California Plastic Products, Inc. Buena Park, California) at the three heights. Volatiles were collected for 3-hr periods. The collections were performed once a week for each set of plants over a four week period for both infested and control plants and for both infestation densities. At the end of each collecting period, the collection filter traps were removed, labeled, wrapped in aluminum foil, and taken to the laboratory for elution.

At the end of the 4 weeks of volatile collection for each of the two sets of plants, all 20 plants that were initially exposed to sawflies were split along the length of the stem to determine if the plants were truly infested. From the total of 20 plants that were initially infested for each set of plants, only five plants from each set were infested. For the analysis of the experiments, only the volatiles collected from these five infested plants from each of the two initial set of plants were used to compare with the uninfested plants at the three different heights, and the two distances, and separately, while growing with uninfested plants in the same pot.

Volatile Elution

After collection, the volatiles were immediately eluted from the volatile collection traps with 200 μl of hexane (Certified A.C.S. Spectranalyzed[®], Fisher Scientific, New Jersey) in a fume hood. The hexane was flash eluted using compressed nitrogen gas (99.9%) and the extract was collected in a 0.2 ml conical glass insert for a 12 x 32 vial (SUN-SRi Chromatography Accessories) inserted into a 1.5 ml clear screw cap vial (Agilent, California). A solution of 10 μl of trans-2-nonene (99%, Aldrich, Milwaukee, Wisconsin) in hexane ($0.73 \text{ ng } \mu\text{l}^{-1}$) was added to each extract as an internal standard and the samples were immediately injected into the GC-MS for analysis.

Volatile Analysis

The volatile extracts were analyzed by coupled gas chromatography-mass spectrometry (GC-MS). The system consisted of an Agilent 6890 Series Gas Chromatograph (Agilent Technologies) fitted with a high resolution gas chromatography column (JandW Scientific HP-5MS, 30 m x 0.25 mm ID, 0.25 μm film, Folsom, California). The mass selective detector was an Agilent 5973 (Agilent Technologies). The temperature program used started at 50 $^{\circ}\text{C}$ and increased 10 $^{\circ}\text{C min}^{-1}$ until it reached 280 $^{\circ}\text{C}$. The chromatograms obtained from the volatiles collected were analyzed with the data analysis program of Agilent Technology's ChemStation software package, using an integration parameter of 13 for all samples. This integration parameter used by the Agilent software, establishes the base line value for the integration or selection of the compound peaks present in a sample. The lower the value of the parameter, the more peaks it selects or integrates. On the other hand, the lower the parameter value, the higher

the presence of contaminants in the selected peaks. The selection of this specific parameter value was based on the general contamination or noise observed in all the samples analyzed.

Statistical Analysis

The volatile collection data obtained from the mass-spectrometer was initially transferred to a Microsoft® Excel spreadsheet where the compounds were quantified by comparison with the internal standard peak area. The amount obtained for each compound was then transformed to the amount produced in one hour.

The infestation status by distance combinations were arranged at random. The three plant heights were treated as repeated measures for the four infestation status by distance combinations. Finally, the four time measurements were considered a second repeated measure for each infestation status by distance by height combination. The experiment was repeated two times using different sets of plants.

Data for the isolated plant experiment were analyzed using a complete factorial structure with experimental group, infested status, distance, height, and time as factors. Random errors included plant within experimental group, plant by infestation status by distance within experimental group, and plant by infestation status by distance by height within experimental group. For the multiple plant experiment, the data was analyzed using the same procedure than for the isolated plant experiment except for the inclusion of the “collection position” variable in the analysis. All analyses were performed using the PROC MIXED procedure with the SAS/STAT (Version 9.2, SAS Institute, 2007).

Results

Isolated Plant Experiments

6-methy-5-heptene-2-one: The collection of this ketone was only significantly affected by individual variables of interest (Table 4.1). Infestation had a significant effect in the collection of this compound ($F = 12.05$, $P = 0.002$). On average, plants infested by the wheat stem sawfly produced $0.44 \text{ ng} \cdot \text{h}^{-1}$ of 6-methy-5-heptene-2-one compared to $0.33 \text{ ng} \cdot \text{h}^{-1}$ produced by uninfested plants (Appendix G). Plant height was significant as well ($F = 128.90$, $P < 0.0001$), greater amounts of this compound were collected from the base of the plant ($0.65 \text{ ng} \cdot \text{h}^{-1}$) compared to the other two heights. Distance from the plant was also significant ($F = 8.46$, $P = 0.008$ with, volatile collections at 0 cm from the plant yielding $0.43 \text{ ng} \cdot \text{h}^{-1}$ of the compound while $0.34 \text{ ng} \cdot \text{h}^{-1}$ was collected at 10 cm from the plant (Appendix G). Time was significant ($F = 2.98$, $P = 0.048$) in the collection of 6-methy-5-heptene-2-one. However, there was no significant interaction between time*infestation ($F = 1.48$, $P = 0.22$). The set of plants from which the volatiles were collected did not have a significant effect in the production of this compound ($F = 1.06$, $P = 0.33$) (Table 4.1).

Interactions between the main variables such as height*distance ($F = 1.65$, $P = 0.2$), height*infestation ($F = 1.49$, $P = 0.23$), distance*infestation ($F = 0.54$, $P = 0.47$), infestation*set of plants ($F = 0.27$, $P = 0.61$), and the combination of height*distance*infestation ($F = 1.90$, $P = 0.16$), were not significant in the collection of 6-methyl-5-heptene-2-one (Table 4.1). Even though the average combined effect of all

three variables was not significant, the analysis of the effect of these variables individually showed that infested wheat plants yielded significantly higher amounts of this compound at the base of the plant and at a distance of 0 cm from the plant ($P = 0.003$) (Figure 4.2a). There was a significant effect of infestation at $\frac{2}{3}$ of the plant height for collections from both 0 cm ($P = 0.0008$) and 10 cm ($P < 0.0001$) from the plant (Figures 4.2a and 4.2b).

The average amounts (ng) of 6-methy-5-heptene-2-one collected from the wheat plants for each independent variable is presented in Appendices G and H.

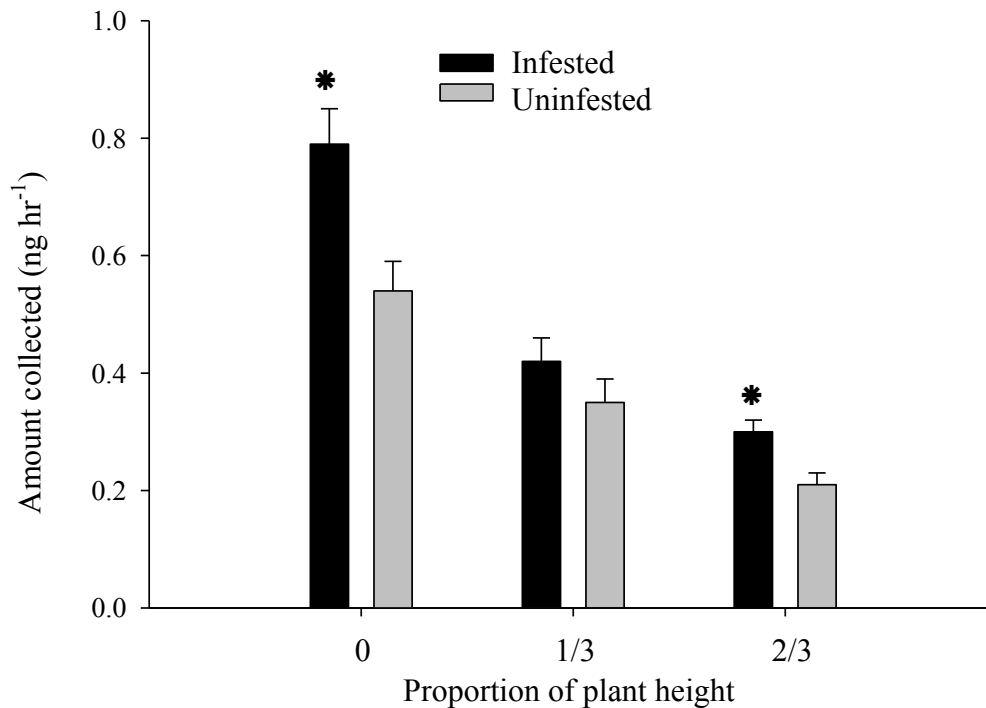


Figure 4.2 a. Effect of height and sawfly infestation on the amount collected ($\pm$ SE) of 6-methyl-5-heptene-2-one adjacent to the main stem (0 cm).

*: Statistically significant ($\alpha = 0.05$).

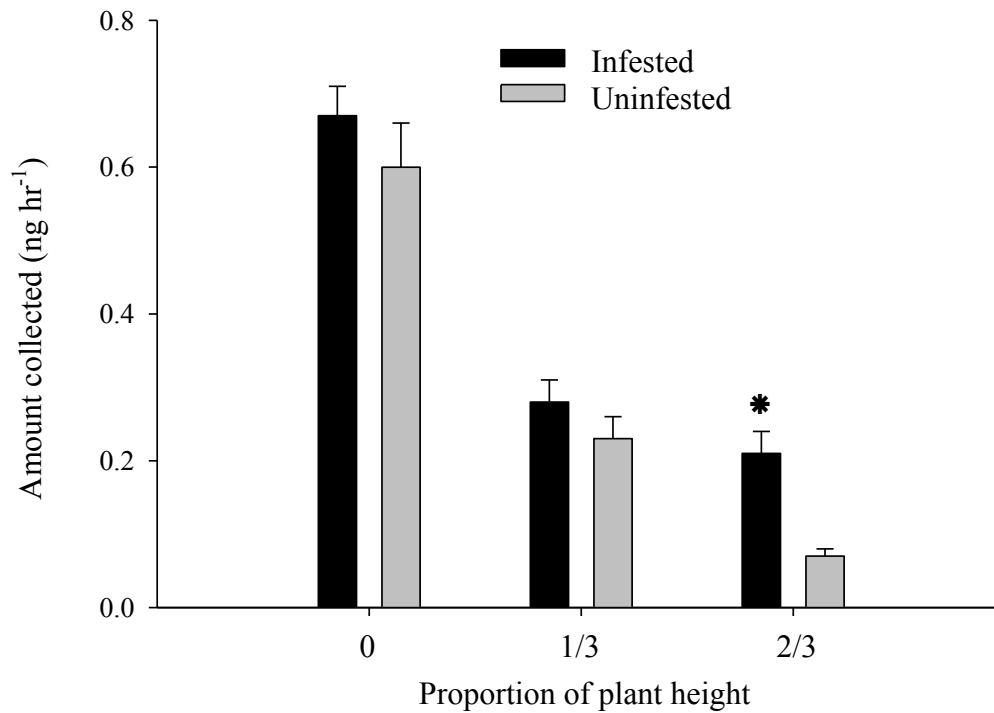


Figure 4.2 b. Effect of height and sawfly infestation on the amount collected ($\pm$ SE) and dispersal of 6-methyl-5-heptene-2-one at 10 cm away from the main stem.

*: Statistically significant ($\alpha = 0.05$).

Table 4.1. Statistical analysis of the effect of independent variables and the interactions between them in the collection of 6-methyl-5-heptene-2-one from the isolated plant experiment.

Variables	<i>F</i>	<i>df</i>	<i>P</i>
Infestation *	12.05	24	0.002
Height *	128.90	64	< 0.0001
Distance *	8.46	24	0.008
Time *	2.98	288	0.048
Plant Set	1.06	8	0.33
Infestation*Height	1.49	64	0.23
Infestation*Distance	0.54	24	0.47
Infestation*Time	1.48	288	0.22
Infestation*Plant Set	0.27	24	0.61
Height*Distance	1.65	64	0.20
Infestation*Height*Distance	1.90	64	0.16

*: Statistically significant ($\alpha = 0.05$).

(Z)-3-hexenyl acetate: The individual variables contributed to the majority of the effects on the collection of this aliphatic compound (Table 4.2). Sawfly infested plants yielded significantly greater amounts of (Z)-3-hexenyl acetate (0.32 ng hr^{-1}) than uninfested plants at only 0.19 ng hr^{-1} ($F = 72.59$, $P < 0.0001$) (Appendix G). Plant height had a significant effect in the collection of this compound ($F = 85.69$, $P < 0.0001$). Greater amounts of (Z)-3-hexenyl acetate were collected at the base of the plants (0.39 ng h^{-1}), compared to the collection at $\frac{1}{3}$ of the plant height (0.24 ng h^{-1}) and at $\frac{2}{3}$ of the plant height (0.13 ng hr^{-1}). Distance from the stem was a significant variable as well ($F = 235.43$, $P < 0.0001$). On average, greater amounts of the compound (0.37 ng hr^{-1}) were collected adjacent to the plant, compared to the 0.14 ng hr^{-1} collected at 10 cm. Even though time was significant in the collection of this compound ($F = 70.98$, $P < 0.0001$), there was no significant effect between time*infestation ($F = 2.02$, $P = 0.11$) (Table 4.2). The factor plant set did not have a significant effect on the collection of this compound ($F = 0.04$, $P = 0.84$). However, significant interactions were detected between height*distance ($F = 14.31$, $P < 0.0001$) and infestation*distance ($F = 25.77$, $P < 0.0001$). For collection of this compound the interaction between infestation*height ($F = 1.22$, $P = 0.30$) as well as the three way interaction infestation*height*distance were not significant ($F = 0.58$, $P = 0.57$) (Table 4.2).

The analysis of the individual variables revealed a significant effect due to sawfly infestation at all collected heights and at both distances for the collection of (Z)-3-hexenyl acetate (Figures 4.3a and 4.3b).

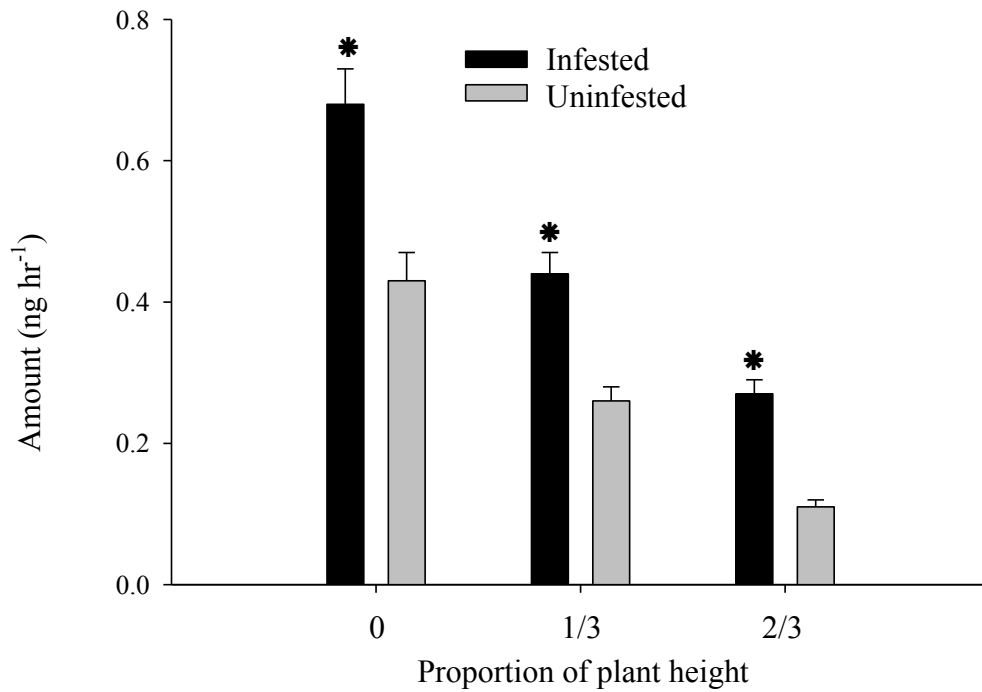


Figure 4.3a. Effect of height and sawfly infestation on the amount collected ($\pm$ SE) of (Z)-3-hexenyl acetate adjacent to the main stem (0 cm). *: Statistically significant ($\alpha = 0.05$).

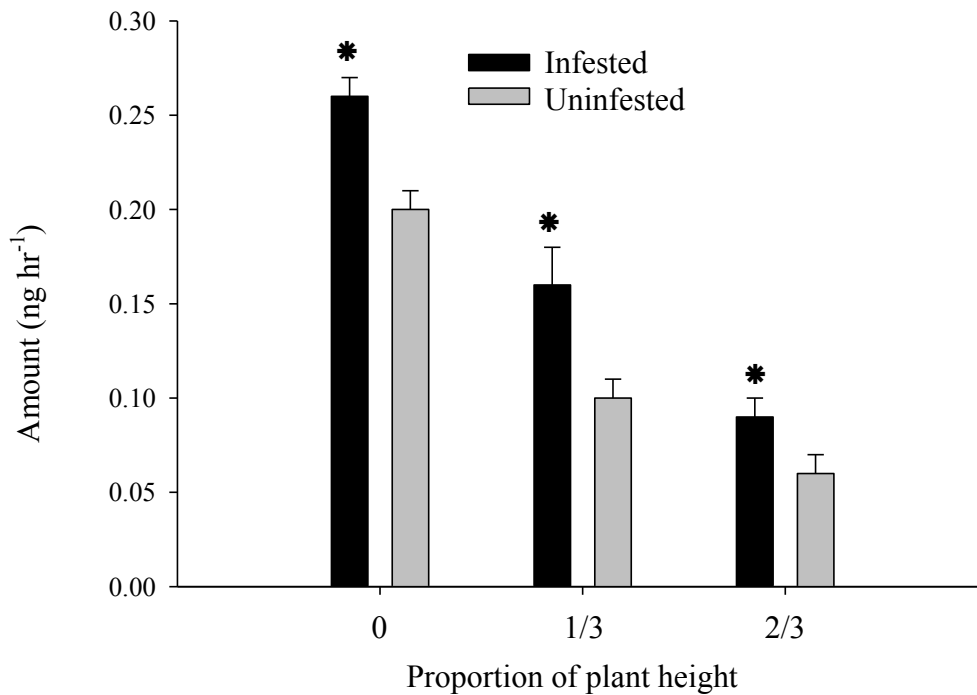


Figure 4.3b. Effect of height and sawfly infestation on the amount collected ($\pm$ SE) and dispersal of (Z)-3-hexenyl acetate at 10 cm away from the main stem.

*: Statistically significant ($\alpha = 0.05$).

The effect of each independent variable on the average amount (ng) of (Z)-3-hexenyl acetate collected from the wheat plants is presented in Appendices G and H.

Table 4.2. Statistical analysis of the effect of independent variables and the interactions between them in the collection of (Z)-3-hexenyl acetate from the isolated plant experiment.

Variables	<i>F</i>	<i>Df</i>	<i>P</i>
Infestation *	72.59	24	< 0.0001
Height *	85.69	64	< 0.0001
Distance *	235.43	24	< 0.0001
Time *	70.98	288	< 0.0001
Plant Set	0.04	8	0.84
Infestation*Height	1.22	64	0.30
Infestation*Distance *	25.77	24	< 0.0001
Infestation*Time	2.02	288	0.11
Infestation*Plant Set	2.33	24	0.14
Height*Distance *	14.31	64	< 0.0001
Infestation*Height*Distance	0.58	64	0.57

*: Statistically significant ($\alpha = 0.05$).

Hexahydrofarnesyl acetone: In the collections of this compound the influence of the main variables infestation ($F = 69.88$, $P < 0.0001$), height ($F = 247.81$, $P < 0.0001$), and distance ($F = 70.77$, $P < 0.0001$) were found to be a significant. Time once again was significant ($F = 39.81$, $P < 0.0001$), but when interacting with infestation it was not ($F = 0.77$, $P = 0.51$) (Table 4.3).

Significantly greater amounts of this compound ($P < 0.0001$) were collected at the base of the plants (2.66 ng hr^{-1}), compared to $\frac{1}{3}$ and $\frac{2}{3}$ of the plants height. The average amount of this compound collected at a distance of 0 cm from the stem (1.86 ng hr^{-1}) was significantly greater ($P < 0.0001$) compared to 1.08 ng hr^{-1} collected at 10 cm from the

stem. On average, significantly lower amounts of this compound ($P < 0.0001$) was produced by sawfly infested plants (1.09 ng hr^{-1}) compared to uninfested plants, which produced 1.85 ng hr^{-1} (Appendix G).

Figures 4.4a and 4.4b show the higher production of hexahydrofarnesyl acetone by uninfested plants at the base and at $\frac{1}{3}$ of the plant height for a collection distance of 0 cm and at all three heights for a distance of 10 cm from the plant.

In the production of this compound there were several interactions that had significant effects, height*distance ($F = 4.88$, $P = 0.01$), infestation*height ($F = 17.67$, $P < 0.0001$), and infestation*distance ($F = 12.62$, $P = 0.002$) (Table 4.3).

The effect of each independent variable on the average amount (ng) of the compound collected from the wheat plants is presented in Appendices G and H.

Table 4.3. Statistical analysis of the effect of independent variables and the interactions between them in the collection of hexahydrofarnesyl acetone from the isolated plant experiment

Variables	<i>F</i>	<i>df</i>	<i>P</i>
Infestation *	69.88	24	< 0.0001
Height *	247.81	64	< 0.0001
Distance *	70.77	24	< 0.0001
Time *	39.81	288	< 0.0001
Plant Set	0.29	8	0.61
Infestation*Height *	17.67	64	< 0.0001
Infestation*Distance *	12.62	24	0.002
Infestation*Time	0.77	288	0.51
Infestation*Plant Set	0.01	24	0.93
Height*Distance *	4.88	64	0.01
Infestation*Height*Distance *	4.00	64	0.02

*: Statistically significant ($\alpha = 0.05$).

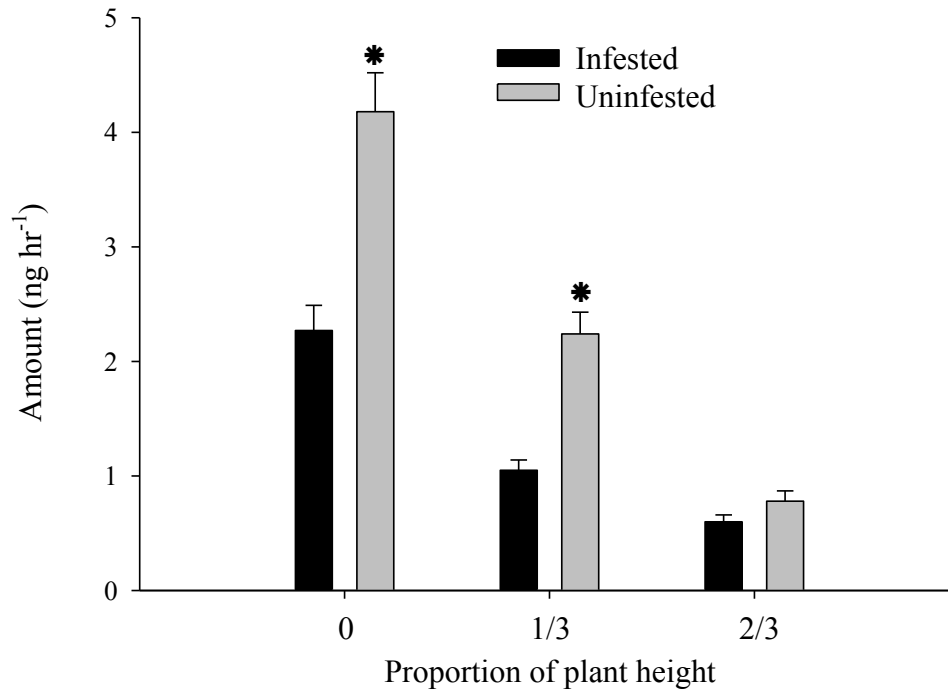


Figure 4.4 a. Effect of height and sawfly infestation on the amount collected ($\pm$ SE) of hexahydrofarnesyl acetone adjacent to the main stem (0 cm).

*: Statistically significant ($\alpha = 0.05$).

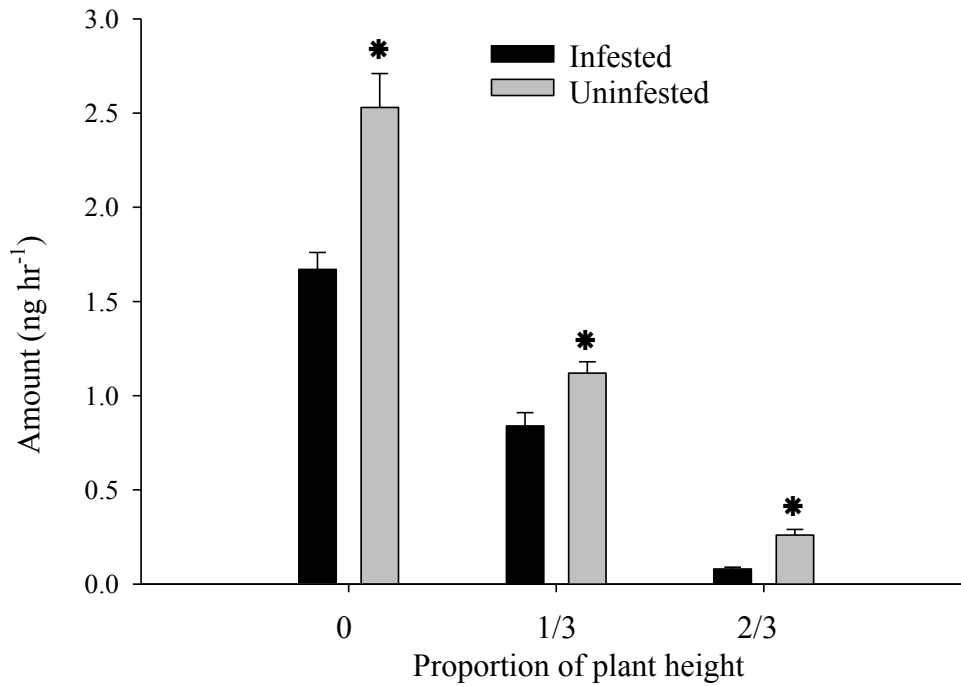


Figure 4.4b. Effect of height and sawfly infestation on the amount collected ($\pm$ SE) and dispersal of hexahydrofarnesyl acetone at 10 cm away from the main stem.

*: Statistically significant ($\alpha = 0.05$).

Multiple Plant Experiments

6-methy-5-heptene-2-one: Sawfly infested pots yielded 0.44 ng hr^{-1} of this compound and uninfested pots yielded only 0.33 ng hr^{-1} infestation, however this difference was not statistically significant ($F = 2.2$, $P = 0.16$). Plant height had a significant effect ($F = 61.03$, $P < 0.0001$) on collection of this ketone. Distance from the plant was also significant ($F = 16.91$, $P = 0.0007$) for the collection of the compound (Appendix G).

Time ($F = 32.51$, $P < 0.0001$) significantly impacted the collection of this compound. However, there was no significant interaction between time*infestation ($F = 2.91$, $P = 0.09$) (Table 4.4). The location where the volatiles were collected from had a significant effect on the amount of this compound ($F = 32.92$, $P < 0.0001$). In the collections made from inside the uninfested group of plants, 0.21 ng hr^{-1} of this compound were collected. In the collections made from the outside of the group of uninfested plants, 0.13 ng hr^{-1} was collected (Appendix H). The set of plants did not have a significant effect ($F = 1.34$, $P = 0.29$)

Interactions between height*distance ($F = 7.81$, $P = 0.001$), height*infestation ($F = 3.55$, $P = 0.03$), and height*collection position ($F = 3.97$, $P = 0.03$) had an effect on the collection of this compound. The combination between the interactions height*distance*infestation ($F = 0.33$, $P = 0.72$) and height*distance*infestation*collection position ($F = 0.26$, $P = 0.77$) were not significant (Table 4.4). The effect of these variables individually was not significant as well, with the exception of sawfly

infested plants collected at $\frac{2}{3}$ of the plant height and at a distance of 0 cm ($P = 0.02$) and at 10 cm ($P < 0.0001$) (Figure 4.5a, 4.5b).

The effect of each independent variable on the average amount (ng) of 6-methy-5-heptene-2-one collected from the wheat plants is presented in Appendices G and H.

Table 4.4. Statistical analysis of the effect of independent variables and the interactions between them in the collection of 6-methyl-5-heptene-2-one from the multiple plant experiment

Variables	<i>F</i>	<i>df</i>	<i>P</i>
Infestation	2.20	18	0.16
Height *	61.03	47	< 0.0001
Distance *	16.91	18	0.0007
Time *	32.51	216	< 0.0001
Collection Position (Cp) *	32.92	18	< 0.0001
Plant Set	1.34	7	0.29
Infestation*Height *	3.55	47	0.04
Infestation*Distance	1.45	18	0.24
Infestation*Time	2.91	216	0.09
Infestation*Plant Set	0.04	18	0.85
Height*Distance *	7.81	47	0.001
Height*Cp *	3.97	47	0.03
Distance*Cp	0.74	18	0.40
Infestation*Height*Distance	0.33	47	0.72

*: Statistically significant ($\alpha = 0.05$).

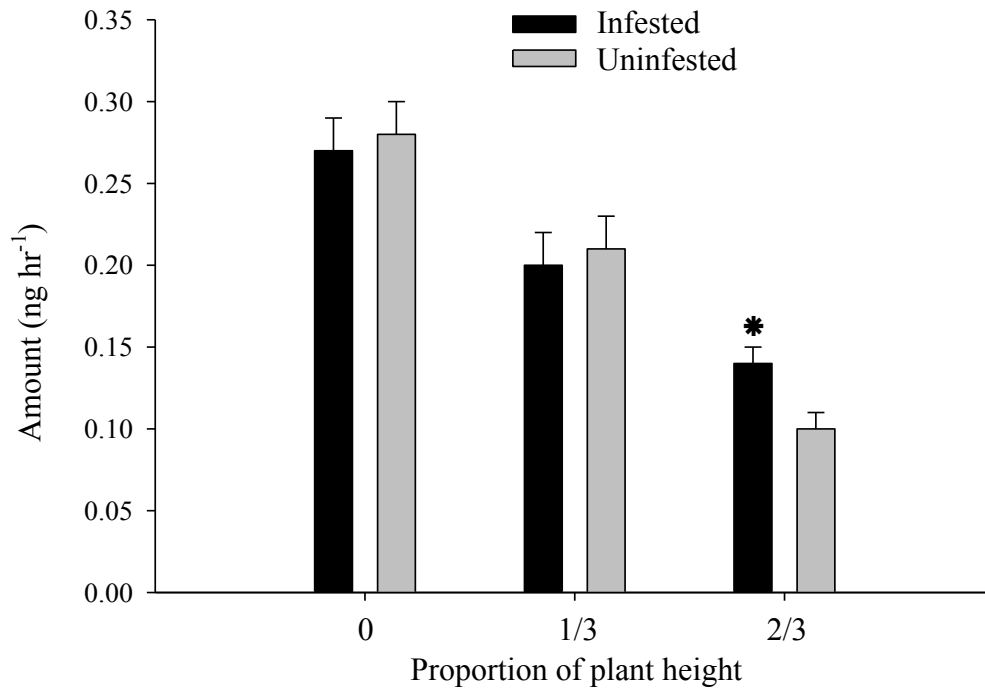


Figure 4.5a. Multiple plant experiment: Effect of height and sawfly infestation on the amount of 6-methyl-5-heptene-2-one collected ($\pm\text{SE}$) adjacent to the main stem (0cm).
*: Statistically significant ($\alpha = 0.05$).

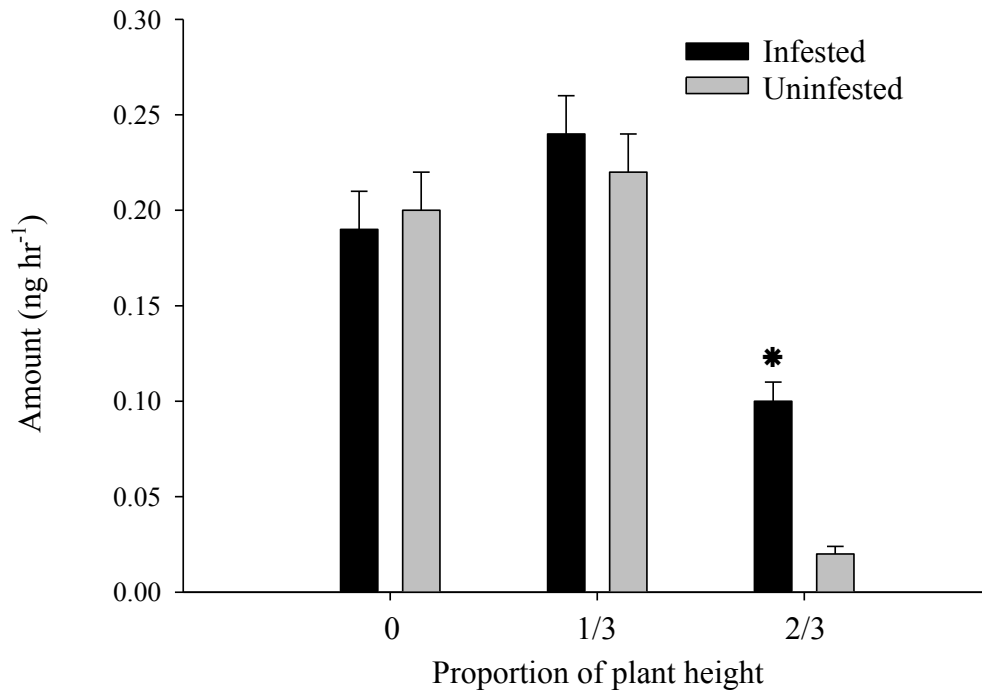


Figure 4.5b. Multiple plant experiment: Effect of height and sawfly infestation on the amount of 6-methyl-5-heptene-2-one ($\pm\text{SE}$) collected at 10 cm away from the main stem.
*: Significant ($\alpha = 0.05$).

(Z)-3-hexenyl acetate: Once again height ($F = 250.80$, $P < 0.0001$), distance ($F = 4.76$, $P = 0.04$), and infestation ($F = 89.38$, $P < 0.0001$) were variables having significant effects on the collection of this compound (Table 4.5). The variable time is significant in the collection of (Z)-3-hexenyl acetate ($F = 100.2$, $P < 0.0001$), but the set of plants is not significant ($F = 1.50$, $P = 0.26$). The collection position is again important in the collection of this compound ($F = 461.79$, $P < 0.0001$). A greater amount of (Z)-3-hexenyl acetate was collected from inside the pots (0.28 ng hr^{-1}) compared to the outside collection (0.14 ng hr^{-1}) (Appendix H).

Significant interactions were found between height*distance ($F = 13.56$, $P < 0.0001$), height*infestation ($F = 10.57$, $P = 0.0002$), distance*infestation ($F = 6.98$, $P = 0.02$), height*collection position ($F = 33.11$, $P < 0.0001$), and distance*collection position ($F = 11.99$, $P = 0.003$).

Figures 4.6a and 4.6b show the effect of height, distance and infestation on the production of this compound. Sawfly infested plants produced higher amounts of this compound at the base of the plant ($P = 0.03$) and at $\frac{1}{3}$ of the height ($P = 0.003$) at a distance of 0 cm and at all three heights at a distance of 10 cm ($P < 0.0001$).

The effect of each independent variable on the average amount (ng) of (Z)-3-hexenyl acetate collected from the wheat plants is presented in Appendices G and H.

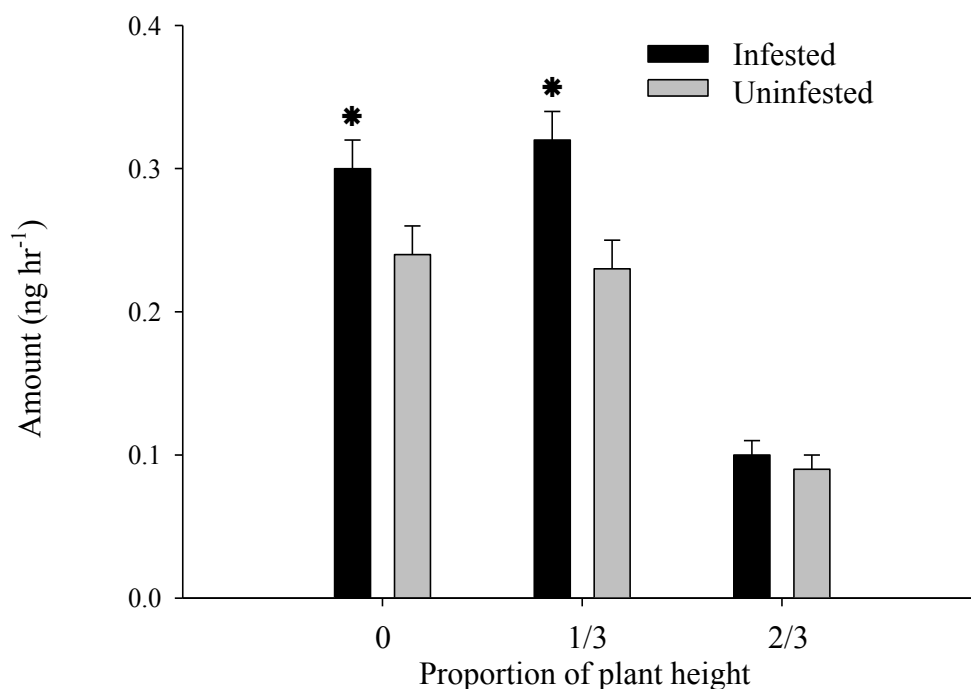


Figure 4.6a. Multiple plant experiment: Effect of height and sawfly infestation on the amount of (Z)-3-hexenyl acetate ($\pm$ SE) collected adjacent to the main stem (0 cm).

*: Significant ($\alpha = 0.05$).

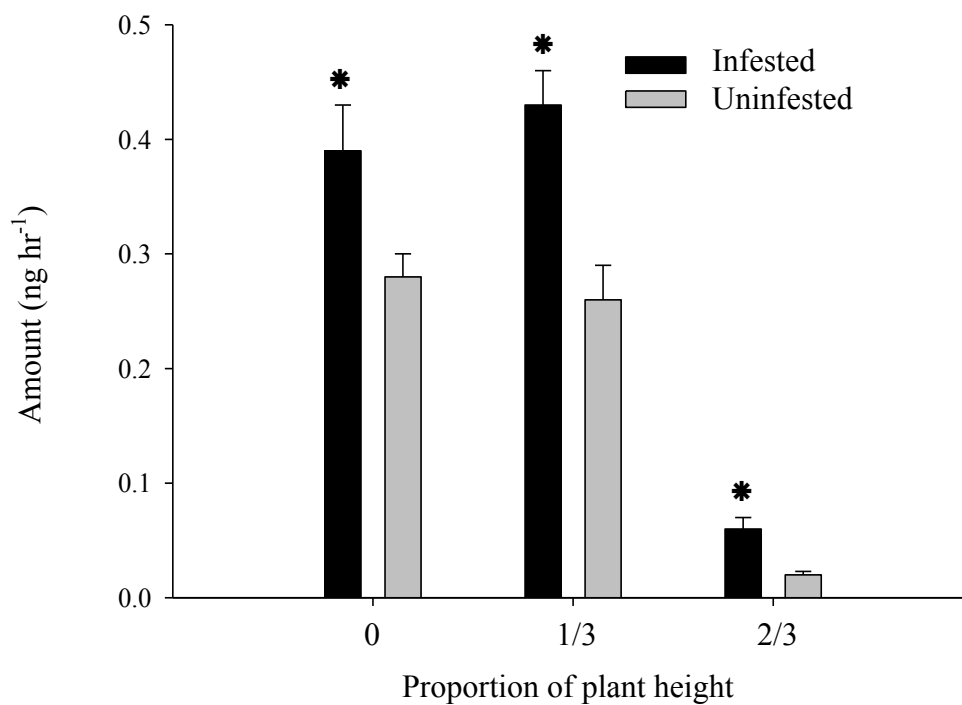


Figure 4.6b. Multiple plant experiment: Effect of height and sawfly infestation on the amount of (Z)-3-hexenyl acetate ($\pm$ SE) collected at 10 cm away from the main stem.

*: Significant ($\alpha = 0.05$).

Table 4.5. Statistical analysis of the effect of independent variables and the interactions between them in the collection of (Z)-3-hexenyl acetate from the multiple plant experiment

Variables	<i>F</i>	<i>df</i>	<i>P</i>
Infestation *	89.38	18	< 0.0001
Height *	250.80	47	< 0.0001
Distance *	4.76	18	0.04
Time *	100.20	216	< 0.0001
Collection Position (Cp) *	461.79	18	< 0.0001
Plant Set	1.50	7	0.26
Infestation*Height *	10.57	47	0.0002
Infestation*Distance *	6.98	18	0.02
Infestation*Time	0.64	216	0.59
Infestation*Plant Set	3.58	18	0.08
Height*Distance *	13.56	47	< 0.0001
Height*Cp *	33.11	47	< 0.0001
Distance*Cp *	11.99	18	0.003
Infestation*Height*Distance	0.53	47	0.59

*: Statistically significant ($\alpha = 0.05$).

Hexahydrofarnesyl acetone: A significant effect caused by plant height ($F = 418.83$, $P < 0.0001$), distance ($F = 5.57$, $P = 0.03$), and infestation ($F = 16.44$, $P = 0.0007$), was also observed in the collection of this compound (Table 4.6). Considerably greater amounts of hexahydrofarnesyl acetone were collected at the base of the wheat plants (1.03 ng hr^{-1}), compared to the lower amounts collected at $\frac{1}{3}$ (0.46 ng hr^{-1}) and $\frac{2}{3}$ (0.14 ng hr^{-1}) of the plant's height. Plants infested by the wheat stem sawfly produced smaller amounts of this compound (0.48 ng hr^{-1}) and the uninfested plants produced greater amounts (0.60 ng hr^{-1}) (Appendix G).

Time also had a significant effect on the production of the compound ($F = 147.08$, $P < 0.0001$), but there was no significant interaction with infestation ($F = 0.58$, $P = 0.63$). As with the previous compounds, the experimental set of plants did not have a significant effect on the collection of this compound ($F = 0.56$, $P = 0.45$), however the collection position was significant ($F = 48.67$, $P < 0.0001$) (Table 4.6).

There were significant interactions between height*distance ($F = 18.12$, $P < 0.0001$), height*infestation ($F = 7.52$, $P = 0.002$), and height*collection position ($F = 58.25$, $P < 0.0001$), but no significant interaction was found between distance*infestation ($F = 0.33$, $P = 0.57$) for the collection of this compound. Figures 4.7a and 4.7b show that only the collections at the base at both distances had a significant effect on the amount of this compound from infested plants ($P = 0.01$ and 0.007)

The effect of each independent variable on the average amount (ng) of hexahydrofarnesyl acetone collected from the wheat plants is presented in Appendices G and H.

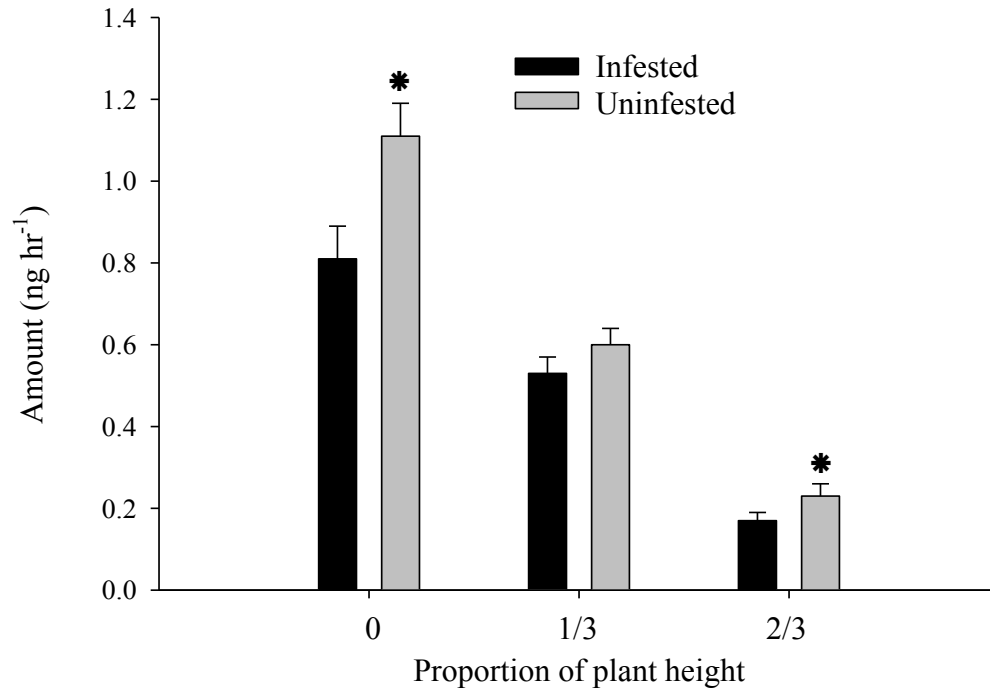


Figure 4.7a. Multiple plant experiment: Effect of height and sawfly infestation on the amount of hexahydrofarnesyl acetone ($\pm$ SE) collected adjacent to the main stem (0 cm).
*: Significant ($\alpha = 0.05$).

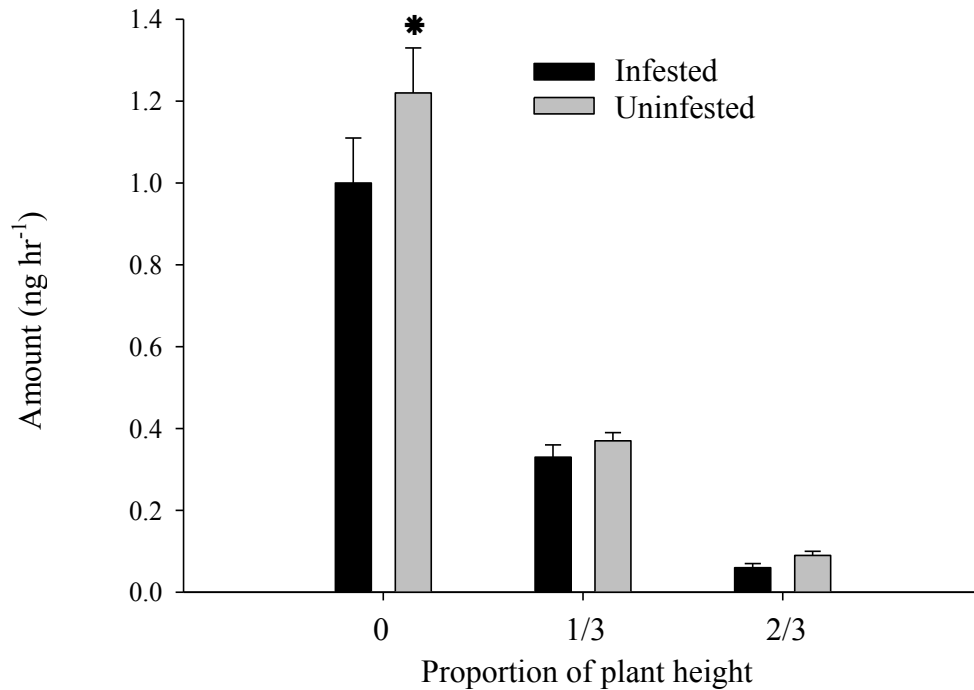


Figure 4.7b. Multiple plant experiment: Effect of height and sawfly infestation on the amount of hexahydrofarnesyl acetone ($\pm$ SE) collected 10 cm away from the main stem.
*: Significant ($\alpha = 0.05$).

Table 4.6. Statistical analysis of the effect of independent variables and the interactions between them in the collection of hexahydrofarnesyl acetone from the multiple plant experiment

Variables	<i>F</i>	<i>df</i>	<i>P</i>
Infestation *	16.44	18	0.0007
Height *	418.83	47	< 0.0001
Distance *	5.57	18	0.03
Collection Position (Cp) *	48.67	18	< 0.0001
Time *	147.08	216	< 0.0001
Plant Set	0.22	7	0.65
Infestation*Height *	7.52	47	0.002
Infestation*Distance	0.33	18	0.57
Infestation*Time	0.58	216	0.63
Infestation*Plant Set	0.21	18	0.65
Height*Cp *	58.25	47	< 0.0001
Distance*Cp	0.45	18	0.51
Height*Distance *	18.12	47	< 0.0001
Infestation*Height*Distance	0.11	47	0.90

*: Statistically significant ($\alpha = 0.05$).

Discussion and Conclusions

In all cases, the amounts of the compounds collected were lesser in the multiple plant groups, perhaps because the plants in these pots were competing for water and nutrients (Gouinguéné and Turlings 2002), even though there is much more plant tissue that could emit volatiles. Interestingly, there were generally still detectable differences between infested and uninfested plants, with greater amounts present at the base of the plant.

Infestation

With the exception of 6-methyl-5-heptene-2-one in the multiple plant group, this most important variable had a significant effect on the collection of all three compounds.

For the isolated group, (Z)-3-hexenyl acetate was always found in significantly greater amounts at all three heights and at both distances in the sawfly infested plants. These results suggest the possibility of parasitoids using this compound as a cue in their host seeking behavior. The parasitoid chemoreceptors might be able to detect the increased amount of (Z)-3-hexenyl acetate to discriminate from the normal production by the uninfested plants to find their hosts. The production of 6-methyl-5-heptene-2-one follows a similar pattern to that for (Z)-3-hexenyl acetate, however, even though there are greater amounts of this compound collected from sawfly infested plants at all heights and distances, only for the collections at the base and at $\frac{2}{3}$ of the plant's height were these values significant. Parasitoids may be using this compound as well but there might be other unknown factors or interactions having an effect in the collection of this compound at $\frac{1}{3}$ of the plant height. Infestation was found to have the opposite effect on hexahydrofarnesyl acetone, higher production from uninfested plants and lower from infested. Nevertheless, it continues to follow the same collection pattern than the other compounds with higher amounts at the base of the plant and at 0 cm from the stem. The higher molecular weight of this compound and the controlled environment with no wind possibly contributed to these results.

Within the multiple plant groups, the production of 6-methyl-5-heptene-2-one was not found to be consistent and significantly higher in sawfly infested plants with the

exception of the collections at $\frac{2}{3}$ of the plant height. Because this is the lightest of the three main compounds, there might be the possibility of an accumulation of it in this within the upper canopy of this multiple group of plants under these experimental conditions. For collection of (*Z*)-3-hexenyl acetate there was an increase in the amounts of this compound collected at $\frac{1}{3}$ of the plant height when the collection occurred 10 cm from the base of the plants. The collection of volatiles from within a group of plants that are close together will probably reveal an increased amount of some of the volatiles produced by the plants because of the reduced space and the number of plants. Even though the compounds analyzed are heavier than air, the presence of multiple plants in a small area might cause an accumulation of some of the lighter compounds at certain heights. The heavier compounds would probably settle faster within the canopy and be found in greater amounts at the base of the plants, which our results showed. Heavier compounds would stay within the canopy of the plants longer, compared to lighter compounds which are be more easily dispersed. Hexahydrofarnesyl acetone, another heavy volatile compound, was found in greater amounts at the base of the multiple plant group, as well.

Hexahydrofarnesyl acetone was much more abundant in collections from the uninfested plants from both experiments. The previously observed pattern of lesser amounts of the compound collected from sawfly infested plants (Peck 2004) was evident.

The overall results obtained on the effect of sawfly infestation on the collection of these compounds suggest that they might be used as host seeking cues by parasitoids to find the wheat stem sawfly.

Plant Height

This variable was found to be significant for the collection of all three compounds for both the isolated and multiple plant groups. This suggests that there is an important effect produced by height on the presence of each of these compounds within a wheat crop.

In the isolated plant group there was a significantly higher amount of all three compounds collected at the base of the wheat plants compared to the other two heights. Because all three of these compounds have a molecular weight heavier than air (6-methyl-5-heptene-2-one = 126 g mol^{-1} , (Z)-3-hexenyl acetate = 142 g mol^{-1} , hexahydrofarnesyl acetone = 268 g mol^{-1} , while air = 28.97 g mol^{-1}), it is possible that these compounds settle at the base of the plants once they are produced and are collected in greater amounts there. The controlled conditions inside the greenhouse where the experiments were performed limit the effect of air currents or other factors as well.

In the multiple plant group there was a similar response pattern for (Z)-3-hexenyl acetate when collected from 0 and 10 cm from the stem and from 6-methyl-5-heptene-2-one when collected at 10 cm from the stem. When collecting volatiles from within a group of plants (at 10 cm) there is an increased collection of some of the volatiles produced by the plants. In these three cases, there was a greater amount of the compound present at $\frac{1}{3}$ of the plant's height. The flag leaves of all secondary stems in an individual pot, which are likely to produce greater amounts of plant volatiles, are located slightly above $\frac{1}{3}$ of the main stem's height. Furthermore, the presence of multiple plants in a small area, without wind or other environmental factors might cause an accumulation of some of the lighter compounds at certain heights of the plants. Taking into account that

all compounds that we are studying are heavier than air, hexahydrofarnesyl acetone is more dense than 6-methyl-5-heptene-2-one and (Z)-3-hexenyl acetate, there is the probability that these compounds descend slowly and can be collected in appreciable numbers by the filters positioned at $\frac{1}{3}$ of the plant height. On the other hand, hexahydrofarnesyl acetone, because it is a larger molecule, accumulated at the base of the plant rapidly and was collected in greater amounts by the filters at this location. This explanation is also supported by the fact that the filters at $\frac{2}{3}$ of the plants height collected the lowest amounts of all three of these compounds.

Distance

As it occurred with plant height, distance had a significant effect in the production of all three of the compounds of interest. Results showed a significantly higher amount of 6-methyl-5-heptene-2-one, (Z)-3-hexenyl acetate, and hexahydrofarnesyl acetone collected at 0 cm from the plant compared to 10 cm. The higher molecular weight of these compounds and the lack of wind and other environmental factors probably limited the dispersion of these compounds. Additional field experiments under natural conditions are needed to establish the dispersal patterns in open settings.

The production of all three compounds in the isolated plant group showed a significantly larger amount collected at 0 cm from the plant and at the base of the plant.

Volatiles collected from the group with multiple plants showed an increase in the amount of some of the compounds collected at 10 cm from the plants. Once again the reduced space and the higher number of plants probably played an important role in these results.

These results suggest that the effect that distance might have in the dispersal of these compounds is probably influenced by other variables such as environmental factors and plant density.

Other Variables

Time was found to be significant for all three compounds at all three heights and both distances. However, the interaction between time and our main variable infestation was not significant for all the compound collections. Because our objective is mainly to establish the effect that sawfly infestation has on the collection of these compounds despite the location of the collection filter, the variable time was averaged and was not considered independently in the analysis.

The collection position was significant in the collection of all three compounds in the multiple plant groups. In these groups, the collection position was a highly significant variable in the amount of the wheat volatiles collected. There was a greater amount of volatiles collected by the filters inside the wheat pots. This suggests that there might be an accumulation of some of the heavier compounds at ground level. Because these volatile compounds are heavier than air, it is possible that undetectable amounts of the molecules reached the filters placed outside the pots. The effect of this variable also supports an effect of plant density on the amount of volatiles present in a specific area.

Variable Interactions

In the production of 6-methyl-5-heptene-2-one from the isolated plant group there were no significant interactions between any of the main variables (Table 4.1). This

suggests that the production of this compound under these conditions is only significantly affected by the main independent variables (Figures 4.2a and 4.2b). For the multiple plant groups there were three significant interactions. One between infestation*distance, caused by the difference in the amount of this compound collected between infested and uninfested plants at the two collection distances. A second interaction, between height*distance due to the difference in the amount of this compound collected between the two distances specifically at the heights of 0 cm and $\frac{1}{3}$ of the plant. The pattern of greater amounts of the compound collected at the lower heights of the plant was modified in the 10 cm collections. There was a greater amount of this compound collected specifically at $\frac{1}{3}$ of the plant's height compared to 0 cm collection. The last interaction, between height*collection position was produced by the inconsistent amount of 6-methyl-5-heptene-2-one collected from the inside and the outside position at the three collection heights. The general pattern of greater amount of this compound collected from the inside position changed at $\frac{1}{3}$ of the plant height. Variation in canopy structure and resource competition probably governs these interactions.

There were two important interactions in the production of (Z)-3-hexenyl acetate from the isolated plant group, infestation*distance and height*distance (Table 4.2). Previous discussion addressed how this compound was collected in significantly greater amounts from sawfly infested plants at 0 cm from the plant compared to 10 cm. There is also an evidently higher amount of this compound collected at the base of the plant and at 0 cm from the stem for $\frac{2}{3}$ of the plant height, which is probably responsible for this interaction (Figures 4.3a and 4.3b).

In the production of (Z)-3-hexenyl acetate from the multiple plant group there were several interactions. There were interactions between infestation*height and infestation*distance which supported the previously reported effects caused by infestation on the production of this compound (Table 4.5). Significantly higher amounts of (Z)-3-hexenyl acetate were collected from sawfly infested plants in a consistent pattern from all three plant heights and both distances. However, a variation in the amount of this compound collected at $\frac{2}{3}$ of the plant's height for both collection distances probably generated these interactions (Figures 4.6a and 4.6b). Interaction between height*collection position and distance*collection position revealed the combined effect that both of these variables had on the production of this compound. Even though, a decrease in the amount of this compound is expected due to dissipation as distance increases, there was a greater amount of (Z)-3-hexenyl acetate collected at the base and at $\frac{1}{3}$ of the plant's height and at 10 cm away from the plants in the inside collections. This variation in pattern is probably responsible for these interactions. The molecular weight of this compound and the presence of uninfested wheat plants at the inside collection site might be responsible for the collection of greater amounts of this compound.

Hexahydrofarnesyl acetone produced by both the isolated group plants and the multiple plant group exhibited the interactions specified in tables 4.3 and 4.6, which can be attributed to the similar pattern obtained for the other two compounds. However, there was an important interaction between infestation*height*distance on the isolated plant group experiment. The significant and substantial differences in the amounts of this compound produced between sawfly infested and uninfested plants at the three collection

heights and the two distances are probably the cause of this complex interaction. There was a greater difference in the amount of hexahydrofarnesyl acetone collected between infested and uninfested plants at the base and at $\frac{2}{3}$ of the plant's height from the collections at a distance of 0 cm and at 10 cm. Specific characteristics of this compound (ripening compound, lower production from infested plants, heavy compound), and the consistent pattern in the production of this compound with well defined differences between infested and uninfested plants suggest the possibility of it being used as a chemical cue in the process of host finding by the sawfly parasitoids.

The ratio of hexahydrofarnesyl acetone to (Z)-3-hexenyl acetate, and to 6-methyl-5-heptene-2-one at the base of the stem was approximately 3:1 for infested plants and 6:1 to 8:1 for uninfested plants respectively; at $\frac{1}{3}$ height the ratio was 2:1 for infested plants vs. 5:1 to 10:1 for uninfested plants; at $\frac{2}{3}$ height the ratio was 2:1 for both infested and uninfested plants. At 10 cm the differences in ratios were less. Host seeking parasitoids could cue on a decreasing gradient of hexahydrofarnesyl acetone as a distant indicator of an infested stem, and subsequently target a decreasing ratio of hexahydrofarnesyl acetone to other compounds.

There is information showing how specialist parasitoids are able to detect very low concentrations of some specific compounds, while generalist parasitoids are not able to detect these concentrations (Smid et al. 2002) and *Bracon cephi* and *B. lissogaster* are highly host specific (Weaver et al. 2005). So, as it occurred with the specialist parasitoid in the Smid et al. (2002) study, these braconids could be cuing in on ratios or very low concentrations of hexahydrofarnesyl acetone while foraging in the plant canopy of the

plants. Turlings et al. (1998) mention the possibility of natural enemies adapting to make good use of rare compounds that are produced by the plants, especially in cases where the host is cryptic and very hard to detect, as is the case for these wheat stem sawfly parasitoids.

Pest infestation has been shown in many publications to be an important factor in the production of plant volatiles (Boevé et al. 1996, Peck 2004). In this study it was found to be a very significant variable in the production of all three of these compounds for both the isolated and the multiple plant groups, which is the key finding of the study. The isolated plant group represents the greatest probability of detection of infestation. However, the multiple plant groups had the combined effect of all 10 plants during the collection process. These results revealed that for all the compounds, the amounts collected were lower in the multiple plant (low infestation density) groups. A probable reason for this is the crowded conditions that greenhouse culture imposes on grouped plants in a pot. The 10 plants in a single pot are subject to additional stresses on volatile production such as water limitation (Takabayashi et al. 1994), crowding, and competition for nutrients (Van Wassenhove et al. 1990), but typically still yielded different amounts when collected from pots containing an infested plant.

To compare these results to the literature it is important to note that publications on induced volatiles involved in host seeking focus on profound changes by looking at unique molecules (Turlings et al. 1990, Mattiacci et al. 1995, Fukushima et al. 2002), but they also have quantitative variation in multiple compounds common to both infested and uninfested plants (Peck 2004). Thus in the absence of unique molecules, it may be that

relative ratios can be used by parasitoids to discriminate larval feeding by stem borers like the wheat stem sawfly, which represent a challenge in the context established for leaf feeding external herbivores.

To further explore this concept, we will collect volatile compounds in the field using the same open system. If cues for infestation are indicated by quantitative variation across an area defined by different collection points, then the same patterns should hold for volatile collections from the canopy in areas that vary in the level of infestation.

References

- Agelopoulos, N. G., K. Chamberlain, and J. A. Pickett. 2000. Factors affecting volatile emissions of intact potato plants, *Solanum tuberosum*: variability of quantities and stability of ratios. *J. Chem. Ecol.* 26:497-511.
- Bengtsson, M., A. C. Bäckman, I. Liblikas, M. I. Ramirez, A. K. Borg-Karlson, L. Ansebo, P. Anderson, J. Löfqvist, and P. Witzgall. 2001. Plant odor analysis of apple: antennal response of codling moth females to apple volatiles during phenological development. *J. Agric. Food Chem.* 49: 3736-3741.
- Beres, B. L., H. A. Cárcamo, and J. R. Byers. 2007. Effect of wheat stem sawfly damage on yield and quality of selected Canadian spring wheat. *J. Econ. Entomol.* 100: 79-87.
- Bruce, T. J. A., L. J. Wadhams and C. M. Woodcock. 2005. Insect host location: A volatile situation. *Trends Plant Sci.* 10: 269-274.
- Buteler, M. 2008. Integrated pest management of the wheat stem sawfly by exploiting semiochemicals to enhance trap crops. [PhD Dissertation]. Entomology, Montana State University, Bozeman, Montana. 218 p.
- Buteler, M., D. K. Weaver, and P. R. Miller. 2008. Wheat stem sawfly-infested plants benefit from parasitism of the herbivorous larvae. *Agric. For. Entomol.* 10: 347-354.
- Dicke, M. and J. A. J. van Loon. 2000. Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomol. Exp. Appl.* 97: 237-249.
- Dudareva, N., E. Pichersky, and J. Gershenzon. 2004. Biochemistry of plant volatiles. *Plant Physiol.* 135: 1893-1902.
- Epsky, N. D. and R. R. Heath. 1998. Exploiting the interactions of chemical and visual cues in behavioral control measures for pest tephritid fruit flies. *Fla. Entomol.* 81: 273-282.
- Fatouros, N. E, M. Dicke, R. Mumm, T. Meiners, and M. Hilker. 2008. Foraging behavior of egg parasitoids exploiting chemical information. *Behav. Ecol.* 19: 677-689.
- Foster, S. P. and M. O. Harris. 1997. Behavioral manipulation methods for insect pest-management. *Annu. Rev. Entomol.* 42:123-146.

- Fukushima, J., Y. Kainoh, H. Honda, and J. Takabayashi. 2002. Learning of herbivore-induced and nonspecific plant volatiles by a parasitoid, *Cotesia kariyai*. *J. Chem. Ecol.* 28: 579-586.
- Godfray, H. C. J. and J. K. Waage. 1988. Learning in parasitic wasps. *Nature* 331: 211.
- Gouinguéné, S. P. and T. C. J. Turlings. 2002. The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiol.* 129: 1296-1307.
- Hansson, B., M. Larsson, and W. Leal. 1999. Green leaf volatile-detecting olfactory receptor neurones display very high sensitivity and specificity in a scarab beetle. *Physiol. Entomol.* 24: 121-126.
- Heath, R. R. and A. Manukian. 1992. Development and evaluation of systems to collect volatile semiochemicals from insects and plant using a charcoal-infused medium for air purification. *J. Chem. Ecol.* 18: 1209-1226.
- Hern, A. and S. Dorn. 2003. Monitoring seasonal variation in apple fruit volatile emissions in situ using solid-phase microextraction. *Phytochem. Anal.* 14: 232-240.
- Hildebrand, D. F., G. C. Brown, D. M. Jackson and T. R. Hamilton-Kemp. 1993. Effects of some leaf-emitted volatile compounds on aphid population increase. *J. Chem. Ecol.* 19: 1875-1887.
- Holmes, N. D. 1977. The effect of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), on the yield and quality of wheat. *Can. Entomol.* 109: 1591-1598.
- Jacobson, M. and M. Beroza. 1963. Chemical insect attractants insects promote their own destruction in responding to traps baited with specific lures. *Science* 140: 1367-1373.
- Knudsen, J. T., L. Tollsten, and L. G. Bergstrom. 1993. Floral scents-a checklist of volatile compounds isolated by head-space techniques. *Phytochemistry* 33: 253-280.
- Konstantopoulou, M. A., F. D. Krokos, and B. E. Mazomenos. 2004. Chemical composition of corn leaf essential oils and their role in the oviposition behavior of *Sesamia nonagrioides* females. *J. Chem. Ecol.* 30: 2243-2256.
- Lerdau, M., A. Guenther, and R. Monson. 1997. Plant production and emission of volatile organic compounds. *BioScience* 47: 373-383.

- Lewis, W. J., R. L. Jones, D. A. Nordlund and H. R. Gross Jr. 1975. Kairomones and their use for management of entomophagous insects: II. Mechanisms causing increase in rate of parasitization by *Trichogramma* spp. J. Chem. Ecol. 1: 349-360.
- Matsui, K. 2006. Green leaf volatiles: hydroperoxide lyase pathway of oxylipin metabolism. Curr. Opin. Plant Biol. 9: 274-280.
- Mattiacci, L., M. Dicke, and M. A. Posthumus. 1995. β -Glucosidase: An elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. Proc. Natl. Acad. Sci. USA 92: 2036-2040.
- Mattiacci, L., B. Ambühl Rocca, N. Scascighini, M. D'Alessandro, A. Hern, and S. Dorn. 2001. Systemically induced plant volatiles emitted at the time of "danger". J. Chem. Ecol. 27: 2233-2252.
- Miyazawa, M., S. Nagai, and T. Oshima. 2008. Volatile components of the straw of *Oryza sativa* L. J. Oleo Sci. 57: 139-144.
- Morrill, W. L., D. K. Weaver, and G. D. Johnson. 2001. Trap strip and field modification for management of the wheat stem sawfly (Hymenoptera: Cephidae). J. Entomol. Sci. 36: 34-45.
- Peck, G. E. 2004. Potential semiochemicals of wheat (*Triticum aestivum* L.) induced by oviposition and feeding of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). [Master's Thesis]. Entomology, Montana State University, Bozeman, Montana. 65 p.
- Pickett, J. A., L. J. Wadhams, and C. M. Woodcock. 1997. Developing sustainable pest control from chemical ecology. Agric. Ecosys. Environ. 64: 149-156.
- Piesik, D., D. K. Weaver, J. B. Runyon, M. Buteler, G. E. Peck, and W. L. Morrill. 2008. Behavioral responses of wheat stem sawflies to wheat volatiles. Agric. For. Entomol. 10: 245-253.
- Schoonhoven, L. M., J. J. A. van Loon, and M. Dicke. 2006. Insect-plant biology. 2nd ed. Oxford University Press, Oxford, New York. Host-plant selection: how to find a host plant. p. 135-168.
- Smid, H. M., J. J. A. van Loon, M.A. Posthumus and L. E. M. Vet. 2002. GC-EAG-analysis of volatiles from Brussels sprouts plants damaged by two species of *Pieris* caterpillars: olfactory receptive range of a specialist and a generalist parasitoid wasp species. Chemoecology 12:169-176.

- Takabayashi, J., M. Dicke and M. A. Posthumus. 1994. Volatile herbivore-induced terpenoids in plant-mite interactions: Variation caused by biotic and abiotic factors. *J. Chem. Ecol.* 20: 1329-1354.
- Tholl, D., W. Boland, A. Hansel, F. Loreto, U. S. R. Röse, and J. Schnitzler. 2006. Practical approaches to plant volatile analysis. *Plant J.* 45: 540-560.
- Tooker, J. F. and C. M. De Moraes. 2007. Feeding by Hessian fly [*Mayetiola destructor* (Say)] larvae does not induce plant indirect defenses. *Ecol. Entomol.* 32: 153-161.
- Turlings, T. C., J. H. Tumlinson, and W. J. Lewis. 1990. Exploitation of herbivore induced plant odors by host-seeking parasitic wasps. *Science* 250: 1251-1253.
- Van Wassenhove, F. A., P. J. Drinck, N. M. Schamp, and G. A. Vulsteke. 1990. Effect of nitrogen fertilizers on celery volatiles. *J. Agric. Food Chem.* 38: 220-226.
- Vet, L. E. M., W. J. Lewis, and R. T. Cardé. 1995. Parasitoid foraging and learning. *In*: Cardé, R. T., and Bell W. J. (Eds.), *Chemical ecology of insects 2*. Chapman and Hall, New York. p. 65-101.
- Vinson, S. B. 1998. The general host selection behavior of parasitoid hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. *Biol. Control.* 11: 79-96.
- 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. *Biol. Control* 34: 1-11.
- Weiss, M. J. and W. L. Morrill. 1992. Wheat stem sawfly (Hymenoptera: Cephidae) revisited. *Am. Entomol.* 38: 241-245.

CHAPTER 5

INFLUENCE OF WHEAT STEM SAWFLY INFESTATION LEVEL ON LOCALIZED
VARIABILITY OF BEHAVIORALLY ACTIVE COMPOUNDS IN WHEAT FIELDSIntroduction

Wheat, *Triticum aestivum* L. (Poales: Poaceae) is one of the most important crops in the world. The United States is the third largest producer of wheat worldwide, producing approximately 150 million tons in 2008 (USDA-NASS 2009). The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae) is the most serious insect pest of wheat in the northern Great Plains of North America, causing losses of tens of millions of dollars each year (Weiss and Morrill 1992, Weaver et al. 2004, 2005). This species is thought to be native to North America (Ainslie 1920, Criddle 1922, Farstad 1940, Weiss and Morrill 1992); but this is under some debate (Ivie and Zinovjev 1996, Ivie 2001). Originally feeding on grasses and having preference for the large stemmed grasses, such as wheatgrass (*Agropyron dasystachyum* (Hook.)), rye (*Secale cereal* L.) and *Bromus* spp. (Ainslie 1920, Criddle 1922), the wheat stem sawfly readily adapted its feeding habits to wheat because the development of farming prompted the rapid transformation of existing feral grass areas into new wheat crops (Farstad 1940, Callenbach and Hansmeier 1945, Davis 1955, Holmes 1978).

The wheat stem sawfly is univoltine. In Montana adults emerge from late May through late June or early July and mate soon after emergence. Females oviposit inside the wheat stem and several females can oviposit in the same stem (Holmes 1982). The

eggs hatch in approximately 7 days and the larva begins to feed first on parenchymous and then on the vascular tissue in the stem (Ainslie 1920, Holmes 1954). This reduces nutrient and water movement in the plant and negatively affects yield and grain quality (Seamans et al. 1944, Wallace and McNeal 1966, Holmes 1977, Weiss and Morrill 1992, Morrill et al. 1994). As the plant begins senescing, the sawfly larva migrates toward the base of the plant where it cuts a notch around the inside of the stem wall causing the wheat plant to lodge when exposed to wind or rain (Criddle 1922, Davis 1955, Holmes 1978, 1982). Sawfly larvae construct a frass plug under the cut notch and spin a cocoon or hibernaculum below the plug where it will remain in diapause until the following year (Villacorta et al. 1971).

Naturally occurring biological control agents have been found to be effective in suppressing sawfly populations in locations with heavy infestations (Holmes et al. 1963, Runyon et al. 2001). Considerable variability in the percentage of parasitism by two species of congeneric braconids has been observed (Morrill et al. 1998), but recent reports show that parasitism rates can be significant enough to be recognized as economically and ecologically desirable (Runyon 2001, Runyon et al. 2002).

Chemical ecology is a relatively new tool that has been found useful in the understanding and management of insect pests. Over the past three decades, an extensive amount of information on the production of volatile compounds by plants has been published (Dudareva et al. 2004, Ode 2006). More recent studies have determined how plants release specific volatile compounds when they are attacked by pests and how pests and biological control agents use plant volatiles to find their hosts (Wickremasinghe and

Van Emdem 1992, McCall et al. 1993, Tumlinson et al. 1993, Röse et al. 1997, Kessler and Baldwin 2001, Ode 2006). However, most of these studies have been performed under laboratory and greenhouse conditions, and limited work has been performed directly in the field (Boeve et al. 1996, Dorn and Hern 1999, Hunter 2002, Rott et al. 2005, Vallat et al. 2005, Vallat and Dorn 2005).

The wheat stem sawfly develops inside the stem lumen and feeds cryptically. The chemical ecology of stem boring in grasses has been studied to very limited extent (Degenhardt, 2009), with the most informative study being one conducted in the laboratory using small maize (*Zea mays* L.) plants that were penetrated by young European corn borer larvae (Turlings et al. 1998). However, the volatiles were collected over the interval that included external herbivory and subsequent boring to enter the stem. Thus, this system has not been fully characterized and little is known about compounds that are involved in host seeking by those parasitoids (Landis and Hass, 1992) that attack larvae feeding inside a maize stalk.

A related research area is the secondary responses to gall forming insects feeding on wheat. In this case, a single study has been conducted and it was concluded that Hessian fly larvae did not induce volatiles while feeding within the wheat leaf sheath (Tooker and De Moraes, 2007). However, the volatiles were collected only five days after potential oviposition, during which the plants passed from the three leaf stage (Zadoks 12) to a four or five leaf stage (Zadoks 13-15), and would have only possibly been fed upon by very tiny, newly-hatched larvae. Given that most species of the highly abundant parasitoids (Bullock et al, 2004) of this pest feed externally on the larvae of this pest, it is possible

that there are secondary compound patterns that could not have been detected in this study (Tooker and De Moraes 2007).

However, preliminary research on wheat stem sawfly infested plants in the greenhouse has indicated compounds that are being produced in different amounts by infested wheat plants (Peck 2004), which might be used by parasitoids to detect sawfly larvae. As has been shown previously in this dissertation, there are electrophysiologically (Chapter 2) and behaviorally active compounds (Chapter 3) that are produced in measureable amounts by the wheat plants.

The ultimate goal of research on biological management of a field crop pest is to be able to understand the system in the field to hopefully enhance it, so the last step in this research project is to confirm the variation in the amount of behaviorally active compounds in field trials and compare this with the results obtained under greenhouse and laboratory conditions. The data obtained from this set of experiments will provide us with information about the distribution and utility of specific compounds important to host seeking by these parasitoids in wheat stem sawfly infested fields.

Objective

To determine if the amount of behaviorally active chemicals collected are different at locations in the field with different levels of sawfly infestation.

Test Hypothesis

Behaviorally active compounds are present in greater amounts in areas of heavy sawfly infestation and in lower amounts in areas of light sawfly infestation.

Materials and Methods

Plant Material and Sawfly Infestation

Field trials were performed in a privately owned wheat field (40 ha) that had a history of sawfly infestations in the locality of Amsterdam (latitude: 45.75 N, longitude: 111.31 W), Gallatin County, Montana, during the summers of 2004 and 2005. Spring wheat of the variety 'McNeal' was planted in April of both years by seeding directly into the fallow without spring tillage at the rate of 67.3 kg/ha with a row spacing of 17.8 cm. This wheat variety is a hollow stem variety very susceptible to wheat stem sawfly infestation (Kushnak and Talbert 2004). The type of cropping system was wheat-fallow (fallow between crop years). The wheat was grown according to local agronomic practices and a fertilizer (60-40-7 nitrogen, phosphorus, and potassium, respectively) was applied at seeding.

Naturally occurring sawflies emerge from diapause in the infested fallow crop from the previous year in the field. The adults fly during an interval lasting approximately one month after flight begins (mid/late May to mid/late June). Parasitoid flight typically commences at the same time as for sawfly, but the first generation of parasitoid females has a three week latent period that must pass before they can parasitize the larger sawfly

larvae (Muesebeck 1953). Thus, volatile collections began approximately at the time corresponding to the first attack of sawfly larvae in the field. To determine relative infestation in the areas where the volatiles were collected, five random 30-cm samples of row were collected and dissected (Runyon et al. 2002) to determine mean infestation levels.

Volatile Collection

Volatile collections were performed for each year from when the wheat plants reached the stage where the inflorescence of the plants had completely emerged (Zadoks 59, Zadoks et al. 1974) and continued until senescence; this represents approximately the last six weeks before the wheat was harvested. Volatiles were collected at these specific plant stages because they correspond to the duration of the parasitoid flight period, and we were interested in determining the type of volatile compounds and the amounts of these present in areas of high and low infestation at that time.

The collection of volatiles in the field was executed with a portable volatile collection system (PVCS) adapted for field conditions. This system is a significant modification of the system originally described by Heath and Manukian (1992), as it collects air directly from the crop canopy. This system was previously described in Chapter 4, “Quantification and spatial distribution of behaviorally active volatiles from sawfly infested and uninfested wheat plants using an open volatile collection system in the greenhouse”.

Before volatile collections began, the wheat field was evaluated to determine the level of sawfly infestation at the edge and in the center of the field. Wheat plants were

randomly cut and split longitudinally in these two specific areas of the field and the percentage of infestation was determined.

The experimental design for this study consisted of twelve volatile collection sites, each with two collection points. The collection sites were distributed randomly with 6 in the first 2 m of the field (high sawfly infestation) and 6 further into the field at 35 m (low sawfly infestation). At each collection site, two wheat plants were randomly selected approximately 2-3 m apart. On each plant, a glass collection filter secured to a plastic rod was placed at approximately the middle height of the plant and within 1 mm of the stem. Using a thin metal wire, each plant was immobilized and fixed into this position to prevent the wind from separating the stem from the tip of the collection filter, which minimizes the effects on the collection of volatiles. For each collection point the airflow rate used was 0.5 l min^{-1} . This low airflow rate will allow the system to collect plant volatile compounds from the immediate vicinity of each plant. For volatile collection the subsequent week, a new location was selected near the collection points from the previous week. This was necessary because the disassembly and movement of the apparatus out of the field caused localized crop damage. There were 4 collection weeks in 2004 and 5 in 2005.

After 4 hr of volatile collection, each glass filter was removed from the system, carefully labeled, individually wrapped in aluminum foil, and sealed inside a plastic bag. Filters were immediately taken to the laboratory and eluted under a fume hood with 200 μl of hexane (Certified A.C.S. Spectranalyzed[®], Fisher Scientific, New Jersey). The hexane was flash-eluted using compressed nitrogen gas (99.9%) and the extract was

collected in a 0.2 ml conical glass insert for a 12 x 32 vial (SUN-SRi Chromatography Accessories, Rockwood, Tennessee) inserted into a 1.5 ml clear screw cap vial (Agilent, California). A solution of 10 μl of trans-2-nonene (99%, Aldrich, Milwaukee, Wisconsin) ($0.73 \text{ ng } \mu\text{l}^{-1}$) in hexane was added to each sample as an internal standard and the vials were carefully labeled. The samples were injected into a coupled gas chromatography-mass spectrometry (GC-MS) system for analysis.

Volatile Analysis

The volatile extracts were analyzed by coupled gas chromatography-mass spectrometry (GC-MS). The system consists of an Agilent 6890 Series Gas Chromatograph (Agilent Technologies) fitted with a high resolution gas chromatography column (J and W Scientific HP-5MS, 30 m x 0.25 mm ID, 0.25 μm film, Folsom, California). The mass selective detector is an Agilent 5973 (Agilent Technologies). The temperature program used starts at 50 $^{\circ}\text{C}$ and increases 10 $^{\circ}\text{C min}^{-1}$ until it reaches 280 $^{\circ}\text{C}$. The chromatograms obtained from the volatiles collected were analyzed with the data analysis program of Agilent Technology's ChemStation software package, using an integration parameter of 14 for all samples.

Statistical Analysis

The volatile collection data obtained from the mass-spectrometer system was transformed from compound area peaks into amounts by comparison to the internal standard. Because the sample size in our data set was relatively small, normality was checked using the Shapiro - Wilk test and the data was normalized using a log

transformation. The transformed data were statistically analyzed for each year (2004 and 2005) by repeated measures analysis of variance (ANOVA) using a general linear model procedure (PROC GLM). All statistical analyses were performed in SAS (Version 9.2, SAS Institute, 2007). The data presented in all the figures is the original untransformed data.

Results

During the summers of 2004 and 2005, volatiles were consistently collected in the field from areas of both heavy and light infestation by the wheat stem sawfly. In 2004, infestation at the field exterior was 81%, while in the interior it was 7% ($F = 97.94$; $df = 1$; $P < 0.0001$). In 2005, the exterior of the field was 87% infested, while the interior was 9% ($F = 223.08$; $df = 1$; $P < 0.0001$). Thus, in both cases infestation was much greater along the field edges. The analysis of the volatile collections of wheat from areas of the field that were heavily infested by the wheat stem sawfly had significantly greater amounts of four compounds and lower amounts of one compound. The analysis for each compound is given below.

6-methyl-5-hepten-2-one

This compound was collected in greater amounts in heavily infested areas in both 2004 ($F = 22.77$; $df = 1$; $P < 0.0001$) and 2005 ($F = 128.42$; $df = 1$; $P < 0.0001$) (Table 5.1). In 2004, in three of the four weekly collections this compound was collected in significantly greater amounts in sawfly heavily infested areas [week 1 ($P = 0.02$), week 2 ($P = 0.16$), week 3 ($P < 0.0001$), and week 4 ($P < 0.0001$)]. In 2005 it was collected in

significantly greater amounts in sawfly heavily infested areas for all five collection weeks [week 1 ($P < 0.0001$), week 2 ($P < 0.0001$), week 3 ($P < 0.0001$), week 4 ($P < 0.0001$), and week 5 ($P = 0.01$)] (Figures 5.1 and 5.2). There was a significant effect ($P < 0.0001$) due to the repeated measure, collection week, for both years. However, the interaction week*infestation was only significant in 2004 ($P = 0.002$) and not in 2005 ($P = 0.4$) (Table 5.1).

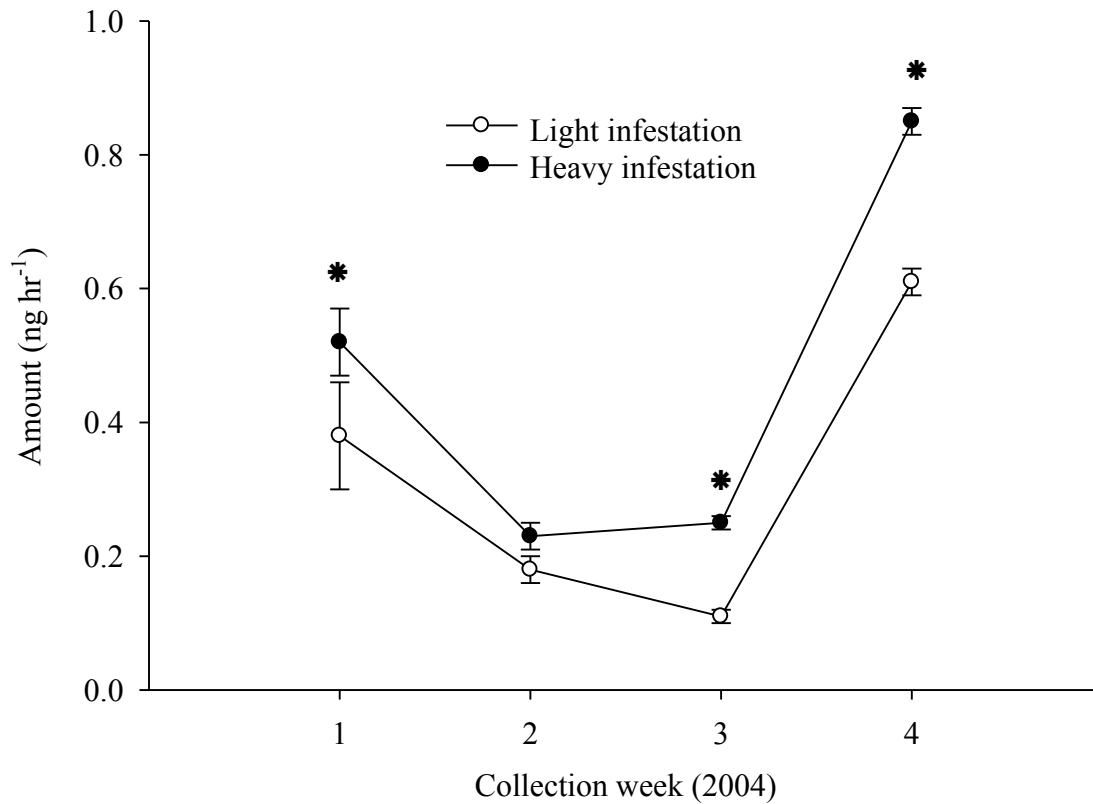


Figure 5.1. Amounts of 6-methyl-5-hepten-2-one ($\pm$ SE) collected in the field from spring wheat plants in areas of heavy and light infestation by wheat stem sawfly in 2004.
*: Statistically significant ($\alpha = 0.05$).

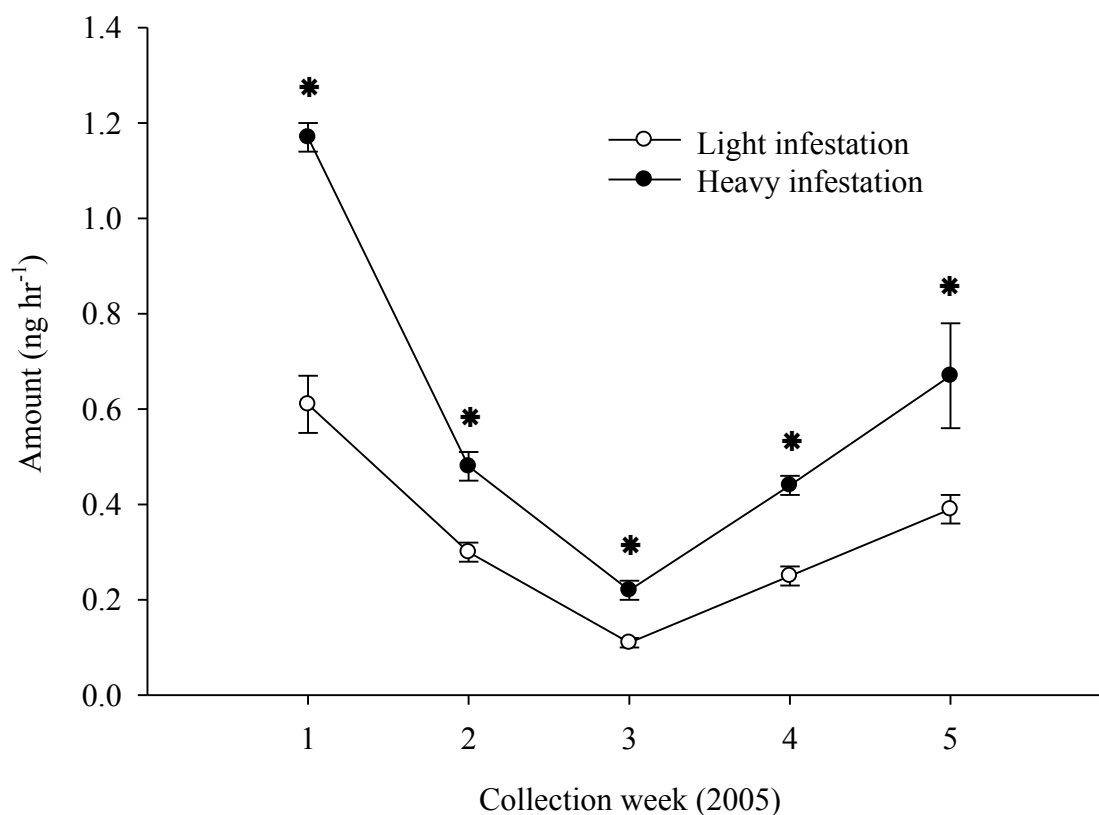


Figure 5.2. Amounts of 6-methyl-5-hepten-2-one ($\pm$ SE) collected in the field from spring wheat plants in areas of heavy and light infestation by wheat stem sawfly in 2005.

*: Statistically significant ($\alpha = 0.05$).

Table 5.1. ANOVA table for field collection of 6-methyl-5-hepten-2-one from spring wheat plants in areas of greater and lesser wheat stem sawfly infestation in 2004 & 2005.

Variables	Year	<i>F</i>	<i>df</i>	<i>P</i>
Week *		228.41	3	< 0.0001
Infestation *	2004	22.77	1	< 0.0001
Week * Infestation *		5.60	3	0.002
Week *		118.47	4	< 0.0001
Infestation *	2005	128.42	1	< 0.0001
Week * Infestation		1.03	4	0.40

*: Statistically significant ($\alpha = 0.05$).

(Z)-3-hexenyl acetate

Significantly greater amounts of (Z)-3-hexenyl acetate were collected from heavily infested areas during both 2004 ($F = 8.5$; $df = 1$; $P = 0.005$) and 2005 ($F = 14.21$; $df = 1$; $P = 0.0003$), when compared to lightly infested areas (Table 5.2). Collection week was a significant repeated measure for both years (2004: $F = 73.26$, $P < 0.0001$; 2005: $F = 11.30$, $P < 0.0001$). However, there were only significant week*infestation interaction effects for 2004 ($F = 5.72$, $P = 0.002$) (Table 5.2). For 2004, (Z)-3-hexenyl acetate was collected in significantly greater amounts in sawfly heavily infested areas for all four collection weeks [week 1 ($P = 0.009$), week 2 ($P = 0.02$), week 3 ($P < 0.0001$), and week 4 ($P < 0.0001$)] (Figures 5.3 and 5.4). Similar results were obtained during 2005 with the exception of the last collection week [week 1 ($P = 0.01$), week 2 ($P = 0.08$), week 3 ($P < 0.0001$), week 4 ($P < 0.0001$), and week 5 ($P = 0.16$)].

Table 5.2. ANOVA table for field collection of (Z)-3-hexenyl acetate from spring wheat plants in areas of greater and lesser wheat stem sawfly infestation in 2004 and 2005.

Variables	Year	<i>F</i>	<i>df</i>	<i>P</i>
Week *	2004	73.26	3	< 0.0001
Infestation *		8.50	1	0.005
Week * Infestation *		5.72	3	0.002
Week *	2005	11.30	4	< 0.0001
Infestation *		14.21	1	0.0003
Week * Infestation		0.78	4	0.54

*: Statistically significant ($\alpha = 0.05$).

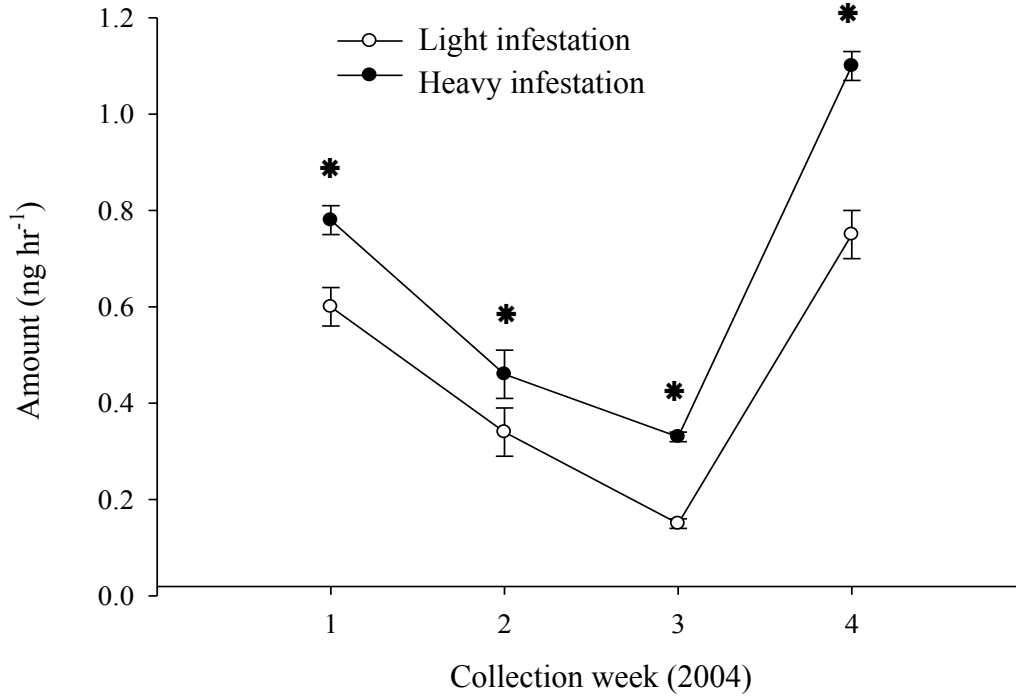


Figure 5.3. Amounts of (Z)-3-hexenyl acetate ($\pm$ SE) collected in the field from spring wheat plants in areas of heavy and light infestation by wheat stem sawfly in 2004.
*: Statistically significant ($\alpha = 0.05$).

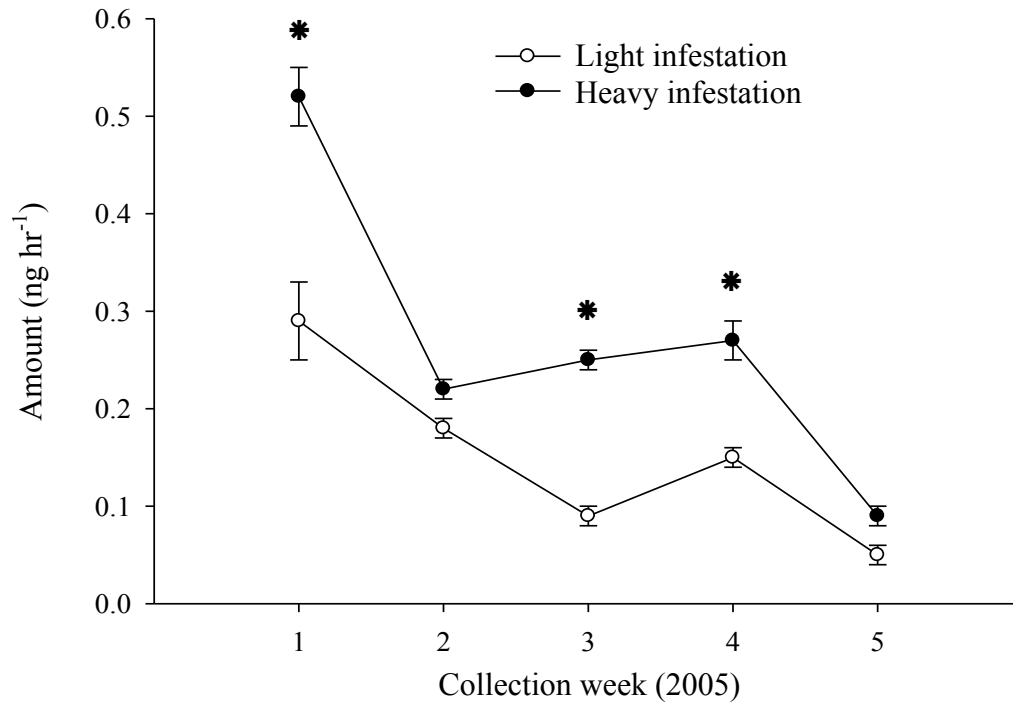


Figure 5.4. Amounts of (Z)-3-hexenyl acetate ($\pm$ SE) collected in the field from spring wheat plants in areas of heavy and light infestation by wheat stem sawfly in 2005.
*: Statistically significant ($\alpha = 0.05$).

Hexahydrofarnesyl acetone

There was a significant difference in the pattern of collections of this compound from heavily infested and lightly infested areas, when compared to all other compounds collected for both years. As opposed to the other compounds, hexahydrofarnesyl acetone was collected in greater amounts in areas of lower infestation during both 2004 ($F = 11.76$; $df = 1$; $P = 0.001$) and 2005 ($F = 36.55$; $df = 1$; $P < 0.0001$) (Figures 5.5 and 5.6) (Table 5.3). Collection week had a significant effect on the amount of this compound collected in both 2004 ($P < 0.0001$) and 2005 ($P = 0.002$). A significant difference in the amount of this compound collected between heavily infested and lightly infested fields was observed only in the later collection weeks of both 2004 [week 1 ($P = 0.32$), week 2 ($P = 0.01$), week 3 ($P = 0.001$), and week 4 ($P = 0.002$)] and 2005 [week 1 ($P = 0.12$), week 2 ($P = 0.24$), week 3 ($P = 0.004$), week 4 ($P < 0.0001$), and week 5 ($P = 0.003$)] (Figures 5.5 and 5.6). The interaction of week*infestation was not significant in either year (2004: $P = 0.30$; 2005: $P = 0.09$) (Table 5.3).

Table 5.3. ANOVA table for field collection of hexahydrofarnesyl acetone from spring wheat plants in areas of greater and lesser wheat stem sawfly infestation in 2004 & 2005.

Variables	Year	<i>F</i>	<i>df</i>	<i>P</i>
Week *		81.12	3	< 0.0001
Infestation *	2004	11.76	1	0.001
Week * Infestation		1.12	3	0.30
Week *		4.58	4	0.002
Infestation *	2005	36.55	1	< 0.0001
Week * Infestation		2.09	4	0.09

*: Statistically significant ($\alpha = 0.05$).

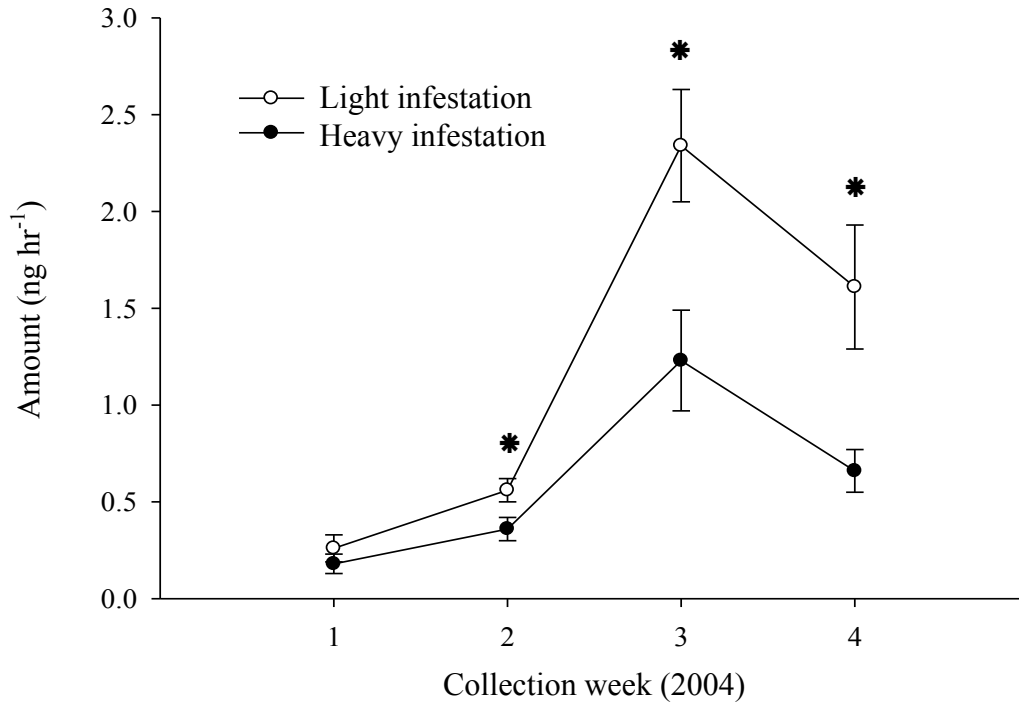


Figure 5.5. Amounts of hexahydrofarnesyl acetone ($\pm$ SE) collected in the field from spring wheat plants in areas of heavy and light infestation by wheat stem sawfly in 2004. *: Statistically significant ($\alpha = 0.05$).

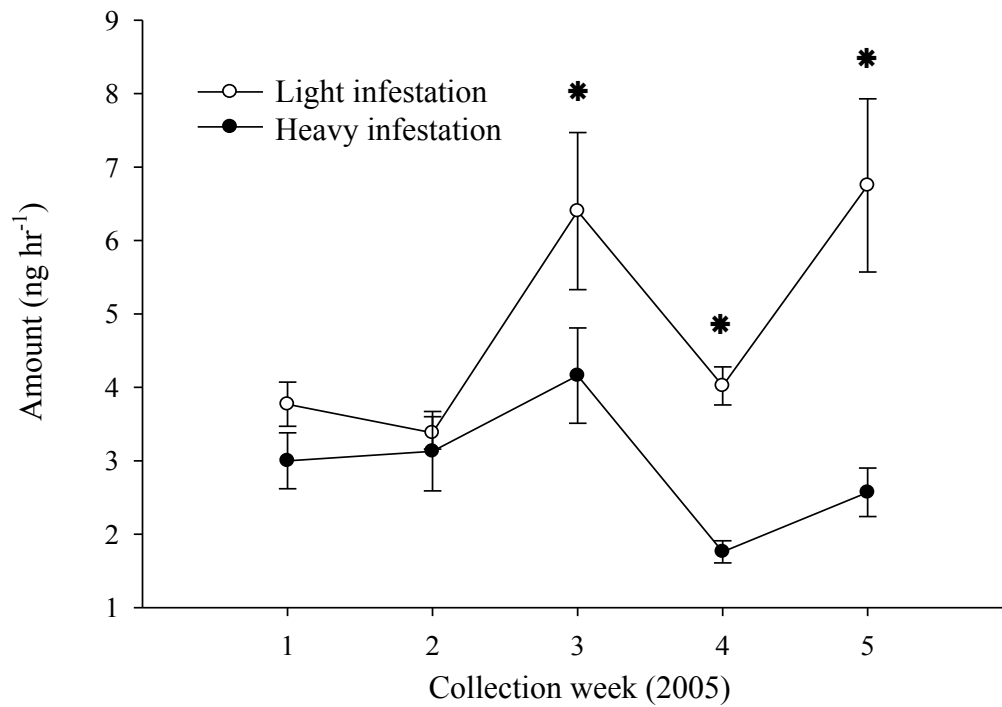


Figure 5.6. Amounts of hexahydrofarnesyl acetone ($\pm$ SE) collected in the field from spring wheat plants in areas of heavy and light infestation by wheat stem sawfly in 2005. *: Statistically significant ($\alpha = 0.05$).

(α)-pinene and Benzaldehyde

Although these compounds are not behaviorally active to the wheat stem sawfly, there was a significant difference found in the amount of α -pinene ($F = 24.73$; $df = 1$; $P < 0.0001$) and benzaldehyde ($F = 20.99$; $df = 1$; $P < 0.0001$) collected between heavily and lightly infested areas during 2005. However, in 2004 this difference was not significant ($P = 0.16$ and $P = 0.72$, respectively). There was a significant difference in the collection of both of these compounds between heavily infested and lightly infested fields for a few of the collection weeks. In 2004, there was a difference in α -pinene in the final collection week [week 4 ($P = 0.0001$)] and during 2005 [week 2 ($P = 0.001$), and week 4 ($P = 0.002$)]. The collection of benzaldehyde was found significantly different only during the first collection weeks in 2005 [week 1 ($P < 0.0001$), and week 2 ($P = 0.003$)]. There was a significant week effect for both years in the collection of these two compounds [α -pinene: $P < 0.0001$ (2004 and 2005); benzaldehyde: $P < 0.0001$ (2004 and 2005)]. The interaction of week*infestation on the collection of α -pinene was not significant for 2004 ($P = 0.94$) and marginally significant for 2005 ($P = 0.07$) (Table 5.4). For benzaldehyde the week*infestation interaction was not significant for 2004 ($P = 0.70$) and significant for 2005 ($P < 0.0001$) (Table 5.4).

Four other compounds that had no behavioral activity were measured in the volatile collections in 2004 and 2005: 2-hexanol, decane, decanal, and nonanal. All four of these compounds were not collected in significantly different amounts between heavily and lightly infested areas. There was no significant effect of the repeated measure, collection week, for any of these compounds.

Table 5.4. ANOVA table for field collection of alpha pinene and benzaldehyde from spring wheat plants in areas of greater and lesser wheat stem sawfly infestation in 2005.

Compound	Variables	<i>F</i>	<i>df</i>	<i>P</i>
(α)-pinene	Week *	61.74	4	< 0.0001
	Infestation *	24.73	1	< 0.0001
	Week * Infestation ♦	2.25	4	0.07
Benzaldehyde	Week *	111.16	4	< 0.0001
	Infestation *	20.99	1	< 0.0001
	Week * Infestation *	25.51	4	< 0.0001

*: Statistically significant ($\alpha = 0.05$). ♦: Statistically significant ($\alpha = 0.01$).

Discussion and Conclusions

As it has been previously reported (Nansen et al. 2005, Weaver et al. 2005) the level of field infestation was much greater on the field periphery. It can be expected that parasitoid abundance follows the abundance of the hosts, as illustrated by Weaver et al. (2005). However, the location for the studies conducted in 2004 and 2005 was unusual in that parasitoids were not detected in either year. The lack of parasitoids provided a unique opportunity to study volatiles associated with herbivory, without the confounding effect of parasitoid altered plant stress (Buteler et al. 2008). Plants are well known to produce a variety of different volatile compounds (Lerdau et al. 1997, Dudareva et al. 2004, Ode 2006). A considerable number of different volatile compounds have been found to be produced by wheat plants, but the number and amount of compounds collected vary by method (Hamilton-Kemp and Andersen 1984, Buttery et al. 1985, Piesik et al. 2008.). Research has shown that many of the volatiles compounds produced by plants are produced under specific conditions. Plants under attack by herbivorous

pests release specific volatile compounds (McCall et al. 1994, Loughring et al. 1995, Paré and Tumlinson 1999, Engelberth et al. 2004, Delphia et al. 2007). Plants produce different volatile compounds during development (Agelopoulos et al. 2000). Plants also vary their production of volatile compounds when environmental conditions change during their development, (Altenburger and Matile 1990, Gouinguené and Turlings 2002). These changes in volatile production caused by variations in environmental conditions have been also documented in wheat (Gianoli and Niemeyer 1996).

Uninfested ‘McNeal’ spring wheat plants release a set of seven volatile compounds, of which four are behaviorally active for females of the wheat stem sawfly (Piesik et al. 2008). Volatiles collected from sawfly infested and uninfested ‘McNeal’ spring wheat plants under controlled greenhouse conditions, revealed that the infested wheat plants produced higher amounts of some compounds compared to uninfested wheat plants uninfested by the sawfly (Peck 2004, Chapter 4: “Quantification and spatial distribution of behaviorally active volatiles from sawfly infested and uninfested wheat plants using an open volatile collection system in the greenhouse”). The volatile collections performed in wheat fields during the summers of 2004 and 2005 corroborated most of the findings of this greenhouse study. Volatiles collected in the field from spring wheat plants indicated greater collections of specific volatiles in areas that were heavily infested by wheat stem sawflies and a reduced collection of another compound. The amount of each compound produced varied over time

6-methyl-5-hepten-2-one

Behavioral studies have shown that this ketone has a repellent effect on adult wheat stem sawflies (Piesik et al. 2008). In field collections of wheat volatiles from locations that were heavily infested by the wheat stem sawfly, there were significantly greater amounts of this compound (Figures 5.1 and 5.2) for most weeks. The consistent pattern of collection of this compound over the two years indicated that the amount of 6-methyl-5-hepten-2-one collected was significantly greater for almost every week from heavily infested areas. Reports on plant defensive mechanisms have found that insect oviposition can induce the production of plant defensive compounds in order to avoid further pest attack (Hilker and Meiners 2006). The current results suggest that this compound might be working as an indirect plant defense mechanism repelling the attack of adult sawflies on wheat plants. In Chapter 2 and Chapter 3, it was shown that this compound is electrophysiologically and behaviorally active for adult braconid parasitoids.

Higher temperatures and low precipitation during the first three collection weeks may have caused a reduction in the release of this compound and a reduction in temperature and increase in precipitation toward the final collection weeks could have helped increase the production (Gouinguéné and Turlings 2002). In addition, the wheat plants are ripening and the insect feeding injury inside the stem increases over time, as well. The variation in the production over the entire collections interval was significant. However, the results indicate that the braconid parasitoids could cue on areas of greater infestation by using the amount of 6-methyl-5-hepten-2-one as an indicator of areas likely to have a higher concentration of hosts.

The significant interaction that was observed only during 2004 for week*infestation could possibly be due to the considerable differences in the amount of this compound produced between sawfly infested and uninfested plants specifically when comparing weeks 1 (infested = 0.52 ng; uninfested = 0.38 ng) and 4 (infested = 0.85 ng; uninfested = 0.61 ng) against weeks 2 (infested = 0.23 ng; uninfested = 0.18 ng) and 3 (infested = 0.25 ng; uninfested = 0.11 ng) (Figure 5.1). There might also be a weather pattern causing this significant interaction. During the last collection week of 2004 there was slight precipitation that did not occur in the previous 3 weeks, which might have had an effect in the elevated production of this compound for that specific week.

(Z)-3-hexenyl acetate

This is a common green leaf volatile compound produced by many species of plants. Behavioral studies have also determined that this compound is an attractant to adult females of the wheat stem sawfly (Piesik et al. 2008). Field collection of volatiles revealed that areas of heavily infested wheat plants released greater amounts of (Z)-3-hexenyl acetate compared to uninfested plants. In both 2004 and 2005 this compound was found to be produced in significantly greater amounts in heavily infested areas for most collection weeks (Figures 5.3 and 5.4).

These results and the behavioral response of the sawfly to this compound indicate that adult wheat stem sawflies are using (Z)-3-hexenyl acetate as a cue in the location of host plants. There is considerable amount of research showing that insects use plant volatile compounds as signals to detect their host plants (Bernays and Chapman 1994, Schoonhoven et al. 1998, Bruce et al. 2005). In this system, (Z)-3-hexenyl acetate

appears to be a compound that is used by the wheat stem sawfly, but may also be a part of the odor cue used by these braconid parasitoids to detect heavily infested areas likely to contain hosts. This compound is electrophysiologically and behaviorally active to adult braconids (Chapter 2 and Chapter 3).

The pattern of collections of this compound from the wheat fields is consistent for each year. During both years, high amounts of this compound were collected in the first week with a decrease in the following weeks and a final increase by week 4 in 2004. High temperatures and low moisture caused by reduced precipitation during weeks 2 and 3 of volatile collection in 2004 could have had an effect on the declining production of this compound by the wheat plants as well, and may have generated the interactions between week and infestation for both years (Table 5.2). However, because (*Z*)-3-hexenyl acetate is a green leaf volatile that is implicated in wound responses (Peck 2004), it is reasonable that the pattern of release will be complicated as green plants experiencing greater injury also start to senesce, as the results of Peck (2004) indicate. There is a general reduction in the collection of this compound (with the exception of week 4 in 2004) as the plants get older and this pattern may also be part of the cues used by the adult braconids.

The significant interaction between week*infestation for this compound in 2004 is very similar to the same interaction for 6-methyl-2-heptene-2-one. The explanation for this interaction could be the same. There were considerable differences in the amount of compound produced between sawfly infested and uninfested plants specifically when comparing weeks 1 (infested = 0.78 ng; uninfested = 0.60 ng) and 4 (infested = 1.10 ng;

uninfested = 0.76 ng) against weeks 2 (infested = 0.46 ng; uninfested = 0.34 ng) and 3 (infested = 0.33 ng; uninfested = 0.15 ng) (Figure 5.1). The precipitation in week 4 could have caused greater production of this compound leading to the significant interaction.

Hexahydrofarnesyl acetone

This compound is known to be a ripening compound (Miyazawa et al. 2008) in wheat plants (Peck 2004). As evidenced by the collections from the portable volatile collection system used in the field, this compound was detected in greater amounts toward the late developmental stages of the wheat plants (Figures 5.5 and 5.6). Collections of this compound over all weeks in both 2004 and 2005 showed a consistent pattern of a general increase in the production of the compound over time, as indicated by the significant effect of the collection week on the amount of this compound collected. However, in contrast with the other compounds, hexahydrofarnesyl acetone was collected in lower amounts from heavily infested areas. This corroborates the results of Peck (2004), who collected wheat volatiles under controlled greenhouse conditions. In addition, Macedo et al. (2005) have observed that physiological stress caused by wheat stem sawfly feeding causes premature senescence. The varying amounts of this compound and the different pattern associated with infestation suggest that this could also be an important cue in locating regions with greater numbers of infested wheat plants.

(α)-pinene and Benzaldehyde

Both of these compounds were present in significantly greater amounts early in 2005 only. The significant variation found in the collection of these compounds during a few

collection weeks did not reflect any specific pattern and it was possibly due to environmental factors occurring during those collection periods. There was no overall difference in the collection of these compounds over the growing season. It is likely that other sources of injury or stress are responsible for this inconsistent and transient response. These compounds are unlikely to be of consistent value in braconid parasitoid host seeking. The significant interaction effect between week*infestation during 2005 for α -pinene was probably caused by the higher relative production of this compound during the initial collection weeks and the considerable reduction toward the last two weeks. Environmental factors probably played an important role in this production pattern based on the precipitation that occurred during the first three weeks of volatile collections during 2005. Benzaldehyde also exhibited a significant interaction between week*infestation for 2005. This compound was collected in an unusually high amount from the sawfly infested plants (5.39 ng) during the first collection week. This substantial difference is probably responsible for the significant interaction between week*infestation. The reason for unusual high production of this compound could be different sources of injury or stress responsible for this inconsistent and transient response.

Other compounds that were collected from these wheat plants in the field (2-hexanol, decane, nonanal, and decanal) were not detected in any significant difference that could play a role in braconid parasitoid host seeking. Other than 2-hexanol, these low molecular weight compounds are common components of leaf epicuticular waxes (Buttery et al. 1985) that were volatilized under high temperatures in the field. These

would be unlikely cues for host seeking by braconid parasitoids. It is also encouraging that the 2-hexanol collections were not significantly different, further indicating the overall patterns of behaviorally active volatile production from heavily infested areas are likely to be governed by specific components that vary quantitatively.

In this system involving volatiles collected in differing amounts in heavily infested areas, we can see that the overall pattern does suggest that there are cues that would allow foraging braconid parasitoids to effectively locate hosts under field conditions. There is little doubt that such compounds will play an important role in the widespread success of these parasitic species. In addition, the results show that if species developing within the stems of grasses cause changes in volatile production, it is not through the release of unique compounds, as has become expected for other herbivorous insects. Given that wheat stem sawfly larvae are cryptic and there is no visible evidence of their presence inside a stem (Weaver et al. 2005, Buteler et al. 2008), the greater release of 6-methyl-5-hepten-2-one and (*Z*)-3-hexenyl acetate by wheat stem sawfly infested plant suggests indirect defenses to sawfly feeding, in addition, the fundamental behavioral responses of the adult sawflies to these compounds (Piesik et al. 2008) suggest several interesting parallels that are also seen in other host-parasitoid systems. A unique signal can also be derived by the more rapid ripening of the infested plants, which corresponds with the flight period of the two most effective parasitoids of the wheat stem sawfly, supports that it is likely that an overall blend of behaviorally and sensorily active compounds are used by the these forging parasitoids. However, further research is needed to fully characterize how this works, and how the exact blend could vary over time.

Secondly, our results show that earlier research on volatile collections from stem-boring or gall forming insects in maize show fundamental differences in the conclusions that can be made. Earlier research (Turlings et al. 1998) focused on the comparison of volatiles from infested maize in comparison to heavy leaf damage caused by caterpillar herbivory. The methods used in the study may not have fully addressed the questions, because the plants used were very young and stem boring herbivory was studied for rapid changes in volatile production, which fits the caterpillar model. It could be that research using feeding caterpillars on the exterior of leaves, which is comparatively easy to study, has led to incorrect conclusions about parasitoid cues from stem boring herbivory, which seems to cause subtle quantitative differences only.

In addition, it is generally viewed that volatile collections should only occur inside sealed chambers, which prevent confounding results from both the failure to detect minor components and by not preventing introduction of compounds arising outside the system being studied. These perceived limitations have resulted in very restricted perceptions about volatile production, and field collections that have been made by other researchers utilize very short collection intervals from highly aromatic plants (Delphia et al. 2007) that are often injured by caterpillars (Turlings et al. 1998). The short collections are used because it is acknowledged that sealed systems create stress on the plants. Wheat releases very small amounts of volatile compounds, and previous research using sealed systems on wheat (Tooker and De Moraes 2007) have been run for a comparatively long time, undoubtedly stressing the plants.

In conclusion, the results obtained from the field collection of wheat plant volatiles using an open collection system determined that collections from spring wheat plants that were heavily infested by wheat stem sawflies produced patterns that could yield odor blends that could be used as cues in host seeking. The patterns were largely consistent with the amounts collected from isolated infested plants versus infested plants at low densities (Chapter 4 “Quantification and spatial distribution of behaviorally active volatiles from sawfly infested and uninfested wheat plants using an open volatile collection system in the greenhouse”) These cues are also consistent with compounds that produce electrophysiological (Chapter 2 “Electroantennogram responses of parasitoids *Bracon cephi* and *Bracon lissogaster* to synthetic wheat volatiles that are released by wheat plants infested with wheat stem sawfly”) and behavioral responses (Chapter 3 “Behavioral responses of the parasitoids *Bracon cephi* and *Bracon lissogaster* to isolated synthetic volatiles produced by wheat plants that are infested by the wheat stem sawfly”) in isolation. Finally, the volatile collection pattern of most of the compounds over the two years were very similar to those reported in preliminary research by Peck (2004) under greenhouse conditions using a closed system confirming the validity of field collections made using the open portable volatile collecting system. Thus the pattern illustrated shows that the conclusions of earlier researchers may be incorrect. Volatile patterns from stem-boring insects, as in this system, are only quantitatively different, perhaps representing a combination of wounding and early ripening. However, there is little doubt that the frequently reported success of many parasitoids that attack cryptic feeding larvae (Turlings et al. 1990, Vet and Dicke 1990, Mattiacci et al. 1999, Mbata et

al. 2004) must involve chemical cues, because unlike external herbivory, the herbivores cannot be seen, nor can acoustic cues be used until the parasitic insect is in contact with the plant. Thus, it is the successful biology of these parasitoid species in the real world that provides the greatest underlying argument for the role of chemical cues, despite the apparent lack of unique, signaling molecules.

References

- Agelopoulos, N. G., K. Chamberlain, and J. A. Pickett. 2000. Factors affecting volatile emissions of intact potato plants, *Solanum tuberosum*: variability of quantities and stability of ratios. *J. Chem. Ecol.* 26:497-511.
- Ainslie, C. N. 1920. The western grass-stem sawfly. U.S. Dept. Agric. Bull. 841. Washington D.C.
- Altenburger, R., and P. Matile. 1990. Further observation on rhythmic emission of fragrance in flowers. *Planta* 180:194-197.
- Bernays, E. A. and R. F. Chapman. 1994. Host-plant selection by phytophagous insects. Chapman and Hall, New York. 312 p.
- Boevé, J., U. Lengwiler, L. Tollesten, S. Dorn, and T. Turlings. 1996. Volatiles emitted by apple fruitlets infested by larvae of the European apple sawfly. *Phytochemistry* 42: 373-381.
- Bruce, T. J. A., L. J. Wadhams, and C. M. Woodcock. 2005. Insect host location: a volatile situation. *Trends Plant Sci.* 10: 269-274.
- Bullock, D. G., N. A. Bosque-Pérez, J. B. Johnson, and F. W. Merickel. 2004. Species Composition and Distribution of Hessian Fly (Diptera: Cecidomyiidae) Parasitoids in Northern Idaho. *J. Kansas Entomol. Soc.* 77: 174-180.
- Buteler, M., D. K. Weaver, and P. R. Miller. 2008. Wheat stem sawfly-infested plants benefit from parasitism of the herbivorous larvae. *Agric. For. Entomol.* 10: 347-354.
- Buttery, R. G., C. Xu, and L. C. Ling. 1985. Volatile components of wheat leaves (and stems): possible insect attractants. *J. Agri. and Food Chem.* 33: 115-117.
- Callenbach, J. A. and M. P. Hansmeier. 1945. Wheat stem sawfly control. Montana Extension Service in Agriculture and Home Economics, Circular No. 164.
- Criddle, N. 1922. The western-stem sawfly and its control. Canadian Department of Agriculture. Pamphlet No. 6, Ottawa. 8 p.
- Davis, E. G. 1955. Status of the wheat stem sawfly in the United States in 1954. *N. D. Agric. Exp. Stn.* 17: 171-175.

- Degenhardt, J. 2009. Indirect defense response to herbivory in grasses. *Plant Physiol.* 149: 96-102.
- Delphia, C. A., M. C. Mescher, and C. M. De Moraes. 2007. Induction of plant volatiles by herbivores with different feeding habits and the effects of induced defenses on host-plant selection by thrips. *J. Chem. Ecol.* 33:997-1012.
- Dorn, S. and A. Hern. 1999. Herbivore-induced volatiles in the apple orchard ecosystem. *IOBC wprs Bull.* 22: 1-5.
- Dudareva, N., E. Pichersky, and J. Gershenzon. 2004. Biochemistry of plant volatiles. *Plant Physiol.* 135: 1893-1902.
- Engelberth, J., H. T. Alborn, E. A. Schmelz, and J. H. Tumlinson. 2004. Airborne signals prime plants against insect herbivore attack. *P. Natl. Acad. Sci.* 101: 1781-1785.
- 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.
- Gianoli, E., and H. M. Niemeyer. 1996. Environmental effects on the induction of wheat chemical defenses by aphid infestation. *Oecologia* 107:549-552.
- Gouinguéné, S. P. and T. C. J. Turlings. 2002. The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiol.* 129: 1296-1307.
- Hamilton-Kemp, T. R., and R. A. Andersen. 1984. Volatile compounds from *Triticum aestivum*. *Phytochemistry* 23: 1176-1177.
- Hartel, K. D., B. A. Frederick, and T. G. Shanower. 2003. Isolation and characterization of microsatellite loci in wheat stem sawfly *Cephus cinctus* and cross-species amplification in related species. *Molec. Ecol. Notes.* 3 p.
- Heath, R. R. and A. Manukian. 1992. Development and evaluation of systems to collect volatile semiochemicals from insects and plant using a charcoal-infused medium for air purification. *J. Chem. Ecol.* 18: 1209-1226.
- Hilker, M. and T. Meiners. 2006. Early herbivore alert: insect eggs induce plant defense. *J. Chem. Ecol.* 32: 1379-1397.
- Holmes, N. D. 1954. Food relations of the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae). *Can. Entomol.* 86: 159-167.

- Holmes, N. D., W. A. Nelson, L. K. Peterson, and C. W. Farstad. 1963. Causes if variation in effectiveness of *Bracon cephi* (Gahan) (Hymenoptera: Braconidae) as a parasite of the wheat stem sawfly. *Can. Entomol.* 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. *Can. Entomol.* 109: 1591-1598.
- Holmes, N. D. 1978. The wheat stem sawfly. *Proc. 26th Annu. Mtg. Entomol. Soc. Alberta*, pp. 2-13.
- Holmes, N. D. 1982. Population dynamics of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), in wheat. *Can. Entomol.* 114: 775-788.
- Hunter, M. D. 2002. A breath of fresh air: beyond laboratory studies of plant volatile-natural enemy interactions. *Agric. For. Entomol.* 4: 81-86.
- Ivie, M. A. and A. G. Zinovjev. 1996. Discovery of the wheat stem sawfly (*Cephus cinctus* Norton) (Hymenoptera: Cephidae) in Asia, with the proposal of a new synonymy. *Can. Entomol.* 128: 347-348.
- Ivie, M. A. 2001. On the geographic origin of the wheat stem sawfly (Hymenoptera: Cephidae): a new hypothesis of introduction from northeastern Asia. *Am. Entomol.* 47: 84-97.
- Kessler, A., and T. Baldwin. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291: 2141-2144.
- Kushnak, G. D., and L. Talbert. 2004. Spring wheat variety notes and comments. Western Triangle Agricultural Research Center, Montana Agricultural Experiment Station, Conrad, MT.
- Landis, D. A. and M. J. Haas. 1992. Influence of landscape structure on abundance and within-field distribution of European corn borer (Lepidoptera: Pyralidae) larval parasitoids in Michigan. *Environ. Entomol.* 21: 409-416.
- Lerdau, M., A. Guenther, and R. Monson. 1997. Plant Production and emission of volatile organic compounds. *BioScience* 47: 373-383.
- Loughrin, J. H., A. Manukian, R. R. Heath, and J. H. Tumlinson. 1995. Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. *J. Chem. Ecol.* 21: 1217-1227.

- Macedo, T. B., R. K. D. Peterson, D. K. Weaver, and W. L. Morrill. 2005. Wheat stem sawfly, *Cephus cinctus* Norton, impact on wheat primary metabolism. *Environ. Entomol.* 34: 719-726.
- Mattiacci, L., E. Hutter, and S. Dorn. 1999. Host location of *Hyssopus pallidus*, a larval parasitoid of the Codling moth, *Cydia pomonella*. *Biol. Control* 15: 241-251.
- Mbata, G. N., S. Shu, T. W. Phillips, and B. Ramaswamy. 2004. Semiochemical cues used by *Pteromalus cerealelle* (Hymenoptera: Pteromalidae) to locate its host, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Ann. Entomol. Soc. Am.* 97: 353-360.
- McCall, P. J., T. C. J. Turlings, J. Loughrin, A. T. Proveaux, and J. H. Tumlinson. 1994. Herbivore-induced volatile emission from cotton (*Gossypium hirsutum* L.) seedlings. *J. Chem. Ecol.* 20: 3039-3050.
- Miyazawa, M., S. Nagai, and T. Oshima. 2008. Volatile components of the straw of *Oryza sativa* L. *J. Oleo Sci.* 57: 139-144.
- 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. *J. Econ. Entomol.* 87: 1373-1376.
- Muesebeck, C. F. W. 1953. Three new reared Braconidae. *Proc. Entomol. Soc. Wash.* 55: 149-151.
- Nansen, C., Weaver, D. K., Sing, S. E., Runyon, J. B., Morrill, W. L., Grieshop, M. J., Shannon, C. L., Johnson, M. L. 2005. Within-field spatial distribution of immature *Cephus cinctus* (Hymenoptera: Cephidae) in Montana wheat fields. *Can. Entomol.* 137: 202-214.
- Ode, P. J. 2006. Plant chemistry and natural enemy fitness: Effects on herbivore and natural enemy interactions. *Annu. Rev. Entomol.* 51:163-85.
- Paré, P. W. and J. H. Tumlinson. 1999. Plant volatiles as a defense against insect herbivores. *Plant Physiol.* 121: 325-331.
- Peck, G. E. 2004. Potential semiochemicals of wheat (*Triticum aestivum* L.) induced by oviposition and feeding of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). [Master's Thesis]. Entomology, Montana State University, Bozeman, Montana. 65 p.

- Pichersky, E. and J. Gershenzon. 2002. The formation and function of plant volatiles: perfumes for pollinator attraction and defense. *Curr. Opin. Plant Biol.* 5: 237-243.
- Piesik, D., D. K. Weaver, J. B. Runyon, M. Buteler, G. E. Peck, and W. L. Morrill. 2008. Behavioral responses of wheat stem sawflies to wheat volatiles. *Agric. For. Entomol.* 10: 245-253.
- Röse, U. S. R., H. T. Alborn, G. Makranczy, W. J. Lewis, and J. H. Tumlinson. 1997. Host recognition by the specialist endoparasitoid *Microplitis croceipes* (Hymenoptera: Braconidae): role of host- and plant-related volatiles. *J. Insect Behav.* 10: 313-329.
- Rott, A. S., J. Häckermann, N. Brand, A. Vallat, and S. Dorn. 2005. Parasitoid exploitation of the seasonal variation in host plant volatile emission for herbivore location. *Entomol. Exp. Appl.* 115: 199-205.
- Runyon, J. B., 2001. Wheat stem sawfly parasitism in varying weed sizes and tillage systems in dryland wheat in Montana. [M.S. thesis], Department of Entomology, Montana State University, Bozeman, Montana. 75 p.
- Runyon, J. B., Morrill, W. L., Weaver, D. K., Miller, P. R. 2002. Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) by *Bracon cephi* and *B. lissogaster* (Hymenoptera: Braconidae) in wheat Welds bordering tilled and untilled fallow in Montana. *J. Econ. Entomol.* 95: 1130-1134.
- Schoonhoven, L. M., T. Jeremy, and J. J. A. van Loon. 1998. Insect-plant biology. From physiology to evolution. Chapman and Hall, London. Chapter 5, Host-plant selection: how to find a host plant. pp 121-153.
- 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 Entomol. Soc. Annu. Rpt.* 75: 10-15.
- Tooker, J. F. and C. M. De Moraes. 2007. Feeding by Hessian fly [*Mayetiola destructor* (Say)] larvae does not induce plant indirect defenses. *Ecol. Entomol.* 32: 153-161.
- Tumlinson, J. H., W. J. Lewis, and E. M. Louise. 1993. How parasitic wasps find their hosts. *Sci. Am.* 264: 100-106.
- Turlings, T. C., J. H. Tumlinson, and W. J. Lewis. 1990. Exploitation of herbivore induced plant odors by host-seeking parasitic wasps. *Science* 250: 1251-1253.

- Turlings, T. C. J., M. Bernasconi, R. Bertossa, F. Bigler, G. Caloz, and S. Dorn. 1998. The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. *Biol. Control* 11:122-129.
- United States Department of Agriculture, National Agricultural Statistics Service. 2009. <http://www.nass.usda.gov/QuickStats/index2.jsp>. (Last Modified: 02/03/2009).
- Vallat, A. and S. Dorn. 2005. Changes in volatile emissions from apple trees and associated response of adult female codling moths over the fruit-growing season. *J. Agric. Food Chem.* 53: 4083-4090.
- Vallat, A., H. Gu, and S. Dorn. 2005. How rainfall, relative humidity and temperature influence volatile emissions from apple trees in situ. *Phytochemistry* 66: 1540
- Vet, L. E. and M. Dicke. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* 37: 141-172.
- Villacorta, A., R. A. Bell, and J. A. Callenbach. 1971. Influence of high temperature and light on postdiapause development of the wheat stem sawfly. *J. Econ. Entomol.* 64:749-751.
- Wallace, L. E., and F. H. McNeal. 1966. Stem sawflies of economic importance in grain crops in the United States. *USDA Tech. Bull.* 1350: 1-50.
- Weaver, D. K., S. E. Sing, J. B. Runyon, and W. L. Morrill. 2004. Potential impact of cultural practices on wheat stem sawfly (Hymenoptera: Cephidae) and associated parasitoids. *J. Agric. Urban. Entomol.* 21: 271-287.
- 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. *Biol. Control* 34: 1-11.
- Weiss, M. J. and W. L. Morrill. 1992. Wheat stem sawfly (Hymenoptera: Cephidae) revisited. *Am. Entomol.* 38: 241-245.
- Wickremasinghe, M. G. V. and H. F. Van Emdem. 1992. Reactions of adult female parasitoids, particularly *Aphidius rhopalosiphi*, to volatile chemical cues from the host plants of their aphid prey. *Physiol. Entomol.* 17: 297-304.
- Zadocks, J. C., T. T. Chang, and C. F. Konzak. 1974. A decimal code for the growth stages of cereals. *Weed Res.* 14: 415-421.

CHAPTER 6

CONCLUSIONS

The present study was intended to provide new information on the chemical ecology involved in the volatile emissions from wheat plants being fed upon by wheat stem sawfly, specifically targeting chemical cues that might be used by congeneric braconid parasitoids to locate infested stems. This information could ultimately provide the basis for the development of more effective control of the wheat stem sawfly based on the interactions between the volatile chemicals produced by sawfly infested wheat plants and the parasitoids *Bracon cephi* and *Bracon lissogaster*.

Electrophysiological studies analyzing the responses of female and male braconid parasitoids antenna to different concentrations of chemical compounds matching those produced by wheat plants infested with wheat stem sawfly, revealed that there are six volatile compounds that generated a positive response from the antenna of these parasitoids at certain concentrations. These compounds were tested for behavioral activity using a wind tunnel to determine their effect on the orientation of adult female and male parasitoids. Three compounds, hexahydrofarnesyl acetone, (Z)-3-hexenyl acetate, and 6-methy-5-heptene-2-one were significantly attractive to both sexes of the parasitoids. However, in both the electrophysiological and behavioral trials females exhibited stronger responses to these compounds, which might be expected based on the need for females to locate the host larvae for oviposition.

This information provides a better understanding of the host seeking behavior of these parasitoids. Because of the cryptic location of the wheat stem sawfly larvae, the ability of these parasitoids to find their hosts must play a very important role in their efficiency as biological control agents. The possibility of knowing the chemical cues used by these parasitoids in finding their hosts can help us improve the understanding of host seeking and potentially enhance control.

However, in order to clarify the role of specific compounds and the active concentrations that the parasitoids detect while host seeking, it is necessary to determine the quantities and the spatial distribution of these compounds that are produced by the wheat plants. In the greenhouse, the collection of volatiles from potted plants at two sawfly infestation densities revealed that greater amounts of volatiles were collected adjacent to the base of isolated infested plants compared to a single infested plant within a patch of 10 plants per pot. These results suggest that competition of the group of plants for water and nutrients affected the collection of volatiles. Greater amounts of volatiles were collected adjacent to the base of the infested stems, suggesting that greater amounts of the behaviorally active compounds might be released at the base of the plant or that they accumulate at the soil surface due to their molecular weight. This greater presence of parasitoid attracting compounds at the base of the plants is supported by previous parasitoid studies in which the majority of parasitoid cocoons found in wheat plants were located in the lower portion of the stem. Hexahydrofarnesyl acetone is an interesting compound that was found in greater amounts in uninfested wheat plants. There was also a decreasing ratio of the amount of this compound produced compared to the other two

behaviorally active compounds, 6-methy-5-heptene-2-one and (*Z*)-3-hexenyl acetate, from the base of the plant to the upper volatile collection point. Host seeking parasitoids could cue on a decreasing gradient of hexahydrofarnesyl acetone, and subsequently on a decreasing ratio of hexahydrofarnesyl acetone to other compounds. There is the possibility of these braconid parasitoids initially targeting very low concentrations of hexahydrofarnesyl acetone while foraging in the plant canopy of the plants.

The final step of this study consisted in performing field trials to determine the difference in volatile production between areas of heavy sawfly infestation and areas of lower infestation. There was a general pattern suggesting that cues that would allow foraging braconid parasitoids to effectively locate hosts under field conditions are more abundant in these areas. Considerably greater amounts of electrophysiologically and behaviorally active compounds were found in areas of heavier sawfly infestation.

Results from this study have important ramifications in the clarification of host seeking by parasitoids of cryptic stem boring insects. The data from the current study show that there are no unique compounds for parasitoids to cue on, as has been repeatedly discovered for systems where the herbivorous hosts are more apparent. This pattern typified by the lack of unique compounds has led to relatively little study of how quantitative variation in the amounts of compounds might result in effective host seeking within the crop canopy where stem borers reside. The current research has provided the foundation for the possible development of improved biological control of the wheat stem sawfly. The identification of the most effective blends used in host seeking could lead to the development of better tools for pest management in this tritrophic system. This could

be accomplished by either artificially augmenting the amounts of active compound blends or even by screening wheat cultivars to determine those that are innately superior in releasing behaviorally active material when infested by wheat stem sawfly larvae.

APPENDICES

APPENDIX A

TOTAL NUMBER OF PARASITIDS USED IN
ELECTROPHYSIOLOGY TESTS

Number of male and female parasitoids for each of the two braconid species that were used with each of the specific compounds tested. Each parasitoid used with a specific compound was subjected to the three concentrations tested (0.01 ng, 100 ng and 10000 ng).

Compound	Females		Males	
	<i>Bracon cephi</i>	<i>Bracon lissogaster</i>	<i>Bracon cephi</i>	<i>Bracon lissogaster</i>
(Z)-3-hexenyl acetate	21	9	2	13
6-methy-5-heptene-2-one	17	5	3	12
Hexahydrofarnesyl acetone	22	6	1	14
(Z)-3-hexanol	14	9	4	11
Benzaldehyde	11	4	2	13
α -pinene	10	5	2	13
(E)-2-hexenyl acetate	13	4	2	13
(E)-2-hexenal	13	7	2	12
(E)- β -ocimene	12	7	2	12
Decanal	12	3	4	11

APPENDIX B

ELECTROPHYSIOLOGICAL RESPONSES OF *B. CEPHI* FEMALES

EAG response ratios of *B. cephi* females to volatile compounds matching those produced by wheat stem sawfly infested wheat plants.

Compounds	N	Conc. (ng)	Ratios	SE	t-test			Regression		
					<i>t</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
(E)-2-hexenal	13	0.01	1.1	0.07	1.12	12	0.29	37.98	1,37	< 0.0001*
		100	1.3	0.19	0.72	12	0.49			
		10000*	22.6	6.87	6.97	12	< 0.0001			
(Z)-3-hexanol	16	0.01	1.2	0.10	0.99	15	0.34	0.19	1,46	0.66
		100	1.1	0.90	0.33	15	0.75			
		10000	1.2	0.15	0.17	15	0.87			
Alpha pinene	10	0.01	0.9	0.10	-1.56	9	0.15	0.64	1,28	0.43
		100	0.9	0.14	-1.29	9	0.23			
		10000	1.0	0.09	0.10	9	0.92			
Benzaldehyde	11	0.01	0.9	0.09	-1.40	10	0.19	9.20	1,31	0.005*
		100	0.9	0.14	-1.88	10	0.09			
		10000*	1.9	0.32	2.60	10	0.03			
6-methyl-5-heptene-2-one	17	0.01	1.2	0.12	1.69	16	0.11	29.57	1,80	< 0.0001*
		100*	1.5	0.12	4.11	16	0.0008			
		10000*	2.0	0.21	5.20	16	< 0.0001			

* : Statistically significant ($\alpha = 0.05$)

EAG response ratios of *B. cephi* females to volatile compounds matching those produced by wheat stem sawfly infested wheat plants.

Compounds	N	Conc. (ng)	Ratios	SE	t-test			Regression		
					<i>t</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
(Z)-3-hexenyl acetate	21	0.01*	2.2	0.40	3.22	20	0.004			
		100*	2.4	0.31	6.32	20	< 0.0001	11.35	1,61	0.001*
		10000*	3.7	0.48	9.28	20	< 0.0001			
(E)-2-hexenyl acetate	13	0.01	1.0	0.11	-0.69	12	0.50			
		100	1.2	0.14	0.62	12	0.55	3.42	1,37	0.07♦
		10000*	1.2	0.12	2.44	12	0.03			
(E)- β -ocimene	12	0.01	1.0	0.08	-1.84	11	0.09			
		100	0.9	0.06	-1.43	11	0.18	0.06	1,34	0.81
		10000	0.9	0.09	-1.83	11	0.09			
Decanal	12	0.01	1.0	0.08	-1.38	11	0.20			
		100	0.9	0.07	-1.30	11	0.22	0.56	1,34	0.46
		10000	1.1	0.19	-0.06	11	0.96			
Hexahydrofarnesyl acetone	22	0.01*	1.8	0.20	5.01	21	< 0.0001			
		100*	1.2	0.08	2.91	21	0.008	36.39	1,64	< 0.0001*
		10000*	0.8	0.07	-2.51	21	0.02			

*: Statistically significant ($\alpha = 0.05$); ♦: Statistically significant ($\alpha = 0.1$).

APPENDIX C

ELECTROPHYSIOLOGICAL RESPONSES OF *B. LISSOGASTER* FEMALES

EAG response ratios of *B. lissogaster* females to volatile compounds matching those produced by wheat stem sawfly infested wheat plants.

Compounds	N	Conc. (ng)	Ratios	SE	t-test			Regression		
					<i>t</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
(E)-2-hexenal	7	0.01	1.0	0.09	-0.58	6	0.58			
		100	1.2	0.14	1.11	6	0.31	33.95	1,19	< 0.0001*
		10000*	16.8	5.42	6.48	6	0.0006			
(Z)-3-hexanol	9	0.01	1.0	0.12	-0.65	8	0.54			
		100	1.0	0.16	-0.49	8	0.64	1.54	1,25	0.23
		10000	1.3	0.16	1.1	8	0.31			
Alpha pinene	5	0.01	1.0	0.08	-0.70	4	0.53			
		100	1.0	0.14	-0.04	4	0.97	0.59	1,13	0.46
		10000	0.9	0.17	-1.15	4	0.32			
Benzaldehyde	4	0.01	0.5	0.17	-2.40	3	0.10			
		100	0.9	0.38	-0.97	3	0.40	6.53	1,10	0.03*
		10000	1.5	0.43	1.30	3	0.28			
6-methyl-5-heptene-2-one	5	0.01	1.1	0.06	1.12	4	0.32			
		100	1.6	0.29	1.61	4	0.18	6.58	1,13	0.02*
		10000*	2.3	0.76	2.81	4	0.04			

*: Statistically significant ($\alpha = 0.05$).

EAG response ratios of *B. lissogaster* females to volatile compounds matching those produced by wheat stem sawfly infested wheat plants.

Compounds	N	Conc. (ng)	Ratios	SE	t-test			Regression		
					<i>t</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
(Z)-3-hexenyl acetate	9	0.01*	2.1	0.51	2.84	8	0.02			
		100*	3.6	1.05	4.68	8	0.002	5.10	1,25	0.03*
		10000*	3.8	0.50	6.46	8	0.0002			
(E)-2-hexenyl acetate	4	0.01	1.0	0.08	-0.30	3	0.78			
		100	1.0	0.10	0.38	3	0.73	6.29	1,10	0.03*
		10000	2.5	0.76	2.17	3	0.12			
(E)- β -ocimene	7	0.01	1.1	0.15	0.14	6	0.90			
		100	0.9	0.13	-1.39	6	0.21	0.01	1,19	0.92
		10000	1.0	0.06	0	6	1.00			
Decanal	3	0.01	1.2	0.10	1.87	2	0.20			
		100	0.9	0.17	-0.59	2	0.62	0.91	1,7	0.37
		10000	1.0	0.09	-0.36	2	0.75			
Hexahydrofarnesyl acetone	6	0.01*	2.0	0.34	3.58	5	0.02			
		100	1.4	0.21	1.75	5	0.14	5.81	1,16	0.03*
		10000	1.0	0.20	-0.53	5	0.62			

*: Statistically significant ($\alpha = 0.05$)

APPENDIX D

ELECTROPHYSIOLOGICAL RESPONSES OF *B. LISSOGASTER* MALES

EAG response ratios of *B. lissogaster* males to volatile compounds matching those produced by wheat stem sawfly infested wheat plants.

Compounds	N	Conc. (ng)	Ratios	SE	t-test			Regression		
					<i>t</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
(E)-2-hexenal	1 3	0.01	1.0	0.07	-0.75	12	0.47	78.65	1,37	< 0.0001
		100	1.2	0.13	0.75	12	0.47			
		10000*	16.7	4.28	12.79	12	< 0.0001			
(Z)-3-hexanol	1 1	0.01	0.9	0.14	-1.72	10	0.12	1.02	1,31	0.32
		100	1.2	0.18	0.27	10	0.79			
		10000	1.3	0.28	0.04	10	0.97			
Alpha pinene	1 3	0.01	1.2	0.08	1.51	12	0.16	0.07	1,37	0.80
		100	1.2	0.13	1.30	12	0.22			
		10000	1.3	0.20	0.96	12	0.35			
Benzaldehyde	1 3	0.01	0.9	0.07	-1.18	12	0.26	2.79	1,37	0.10
		100	1.1	0.06	0.85	12	0.41			
		10000	1.2	0.17	0.95	12	0.36			
6-methyl-5-heptene-2-one	1 2	0.01	1.0	0.08	0.26	11	0.80	11.2	1,34	0.002*
		100*	1.1	0.06	2.60	11	0.03			
		10000*	1.5	0.15	3.87	11	0.003			

* : Statistically significant ($\alpha = 0.05$).

EAG response ratios of *B. lissogaster* males to chemical compounds matching those produced by wheat stem sawfly infested wheat plants.

Compounds	N	Conc. (ng)	Ratios	SE	t-test			Regression		
					<i>t</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
(Z)-3-hexenyl acetate	13	0.01	1.1	0.06	0.52	12	0.61	7.96	1,37	0.008*
		100*	1.3	0.12	2.47	12	0.03			
		10000*	1.6	0.14	3.57	12	0.004			
(E)-2-hexenyl acetate	13	0.01*	0.8	0.08	-2.15	12	0.05	9.06	1,37	0.005*
		100	1.0	0.06	-0.49	12	0.63			
		10000*	1.1	0.05	3.05	12	0.01			
(E)- β -ocimene	13	0.01	0.9	0.09	-1.61	12	0.13	0.76	1,37	0.39
		100	0.9	0.07	-1.90	12	0.08			
		10000	1.0	0.08	-0.86	12	0.41			
Decanal	11	0.01	1.1	0.08	0.92	10	0.38	0.04	1,31	0.84
		100	0.9	0.09	-2.02	10	0.07			
		10000	1.1	0.11	0.42	10	0.68			
Hexahydrofarnesyl acetone	14	0.01*	2.3	0.37	4.63	13	0.0005	13.19	1,40	0.0008*
		100*	1.8	0.28	3.67	13	0.003			
		10000	1.1	0.14	-0.05	13	0.93			

* : Statistically significant ($\alpha = 0.05$).

APPENDIX E

ELECTROPHYSIOLOGICAL RESPONSES OF *B. CEPHI* MALE

EAG response ratios of *B. cephi* males to volatile compounds matching those produced by wheat stem sawfly infested wheat plants.

Compounds	N	Conc. (ng)	Ratios	SE	t-test			Regression		
					<i>t</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
(E)-2-hexenal	2	0.01	0.9	0.00	--	--	--			
		100	1.4	0.35	--	--	--	--	--	--
		10000	10.6	3.95	--	--	--			
(Z)-3-hexanol	4	0.01	0.8	0.15	-1.28	3	0.29			
		100	1.1	0.10	1.05	3	0.37	1.77	1,10	0.21
		10000	1.2	0.26	0.34	3	0.75			
Alpha pinene	2	0.01	1.2	0.35	--	--	--			
		100	1.0	0.20	--	--	--	--	--	--
		10000	0.7	0.10	--	--	--			
Benzaldehyde	2	0.01	1.0	0.05	--	--	--			
		100	1.1	0.15	--	--	--	--	--	--
		10000	0.8	0.20	--	--	--			
6-methyl-5-heptene-2-one	3	0.01	1.3	0.20	1.29	2	0.33			
		100	1.1	0.19	0.04	2	0.97	5.33	1,2	0.15
		10000	1.3	0.12	2.72	2	0.11			

*: Statistically significant ($\alpha = 0.05$)

EAG response ratios of *B. cephi* males to volatile compounds matching those produced by wheat stem sawfly infested wheat plants.

Compounds	N	Conc. (ng)	Ratios	SE	t-test			Regression		
					<i>t</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
(Z)-3-hexenyl acetate	2	0.01	1.0	0.15	--	--	--			
		100	1.3	0.15	--	--	--	--	--	--
		10000	1.4	0.25	--	--	--			
(E)-2-hexenyl acetate	2	0.01	0.9	0.15	--	--	--			
		100	1.2	0.00	--	--	--	--	--	--
		10000	1.4	0.05	--	--	--			
(E)- β -ocimene	2	0.01	0.8	0.10	--	--	--			
		100	1.1	0.05	--	--	--	--	--	--
		10000	1.1	0.15	--	--	--			
Decanal	4	0.01	0.9	0.10	-0.69	3	0.54			
		100	1.0	0.10	0.22	3	0.84	0.05	1,10	0.84
		10000	1.0	0.25	-0.44	3	0.69			
Hexahydrofarnesyl acetone	1	0.01	1.1	0.00	--	--	--			
		100	0.9	0.00	--	--	--	--	--	--
		10000	0.8	0.00	--	--	--			

*: Statistically significant ($\alpha = 0.05$)

APPENDIX F

TOTAL NUMBER OF PARASITIDS USED IN BEHAVIORAL TRIALS

Number of male and female parasitoids from each of the two braconid species that were used in the behavioral trials with each of the specific compounds tested.

Compound	Con. (μ g)	Females		Males	
		<i>Bracon cephi</i>	<i>Bracon lissogaster</i>	<i>Bracon cephi</i>	<i>Bracon lissogaster</i>
(Z)-3-hexenyl acetate	90	85	15	2	98
6-methy-5-heptene- 2-one	35	77	23	5	95
hexahydrofarnesyl acetone	0.3	89	11	0	100
	9	92	8	3	97
(Z)-3-hexanol	423	81	19	12	88
α -pinene	48	80	20	7	93
(E)-2-hexenyl acetate	1.4	79	21	3	97
(E)-2-hexenal	423	88	12	3	97
(E)- β -ocimene	215	71	29	6	94

APPENDIX G

EFFECT OF HEIGHT, DISTANCE, AND INFESTATION ON
WHEAT VOLATILE COLLECTION

Effect of height, distance, and infestation on the average amount (ng) of volatile compounds collected from the isolated plant and multiple plant experiments.

Compounds	Isolated Plants					
	Height					
	0	SE	$\frac{1}{3}$	SE	$\frac{2}{3}$	SE
6-methyl-5-heptene-2-one	0.65	0.03	0.32	0.02	0.20	0.01
(Z)-3-hexenyl acetate	0.39	0.02	0.24	0.01	0.13	0.01
Hexahydrofarnesyl acetone	2.66	0.13	1.31	0.07	0.43	0.04

Compounds	Multiple Plants					
	Height					
	0	SE	$\frac{1}{3}$	SE	$\frac{2}{3}$	SE
6-methyl-5-heptene-2-one	0.23	0.01	0.22	0.01	0.09	0.01
(Z)-3-hexenyl acetate	0.30	0.01	0.31	0.01	0.07	0.01
Hexahydrofarnesyl acetone	1.03	0.05	0.46	0.02	0.14	0.01

Compounds	Isolated Plants			
	Distance			
	0 cm	SE	10 cm	SE
6-methyl-5-heptene-2-one	0.43	0.02	0.34	0.02
(Z)-3-hexenyl acetate	0.37	0.02	0.14	0.01
Hexahydrofarnesyl acetone	1.86	0.11	1.08	0.07

Compounds	Multiple Plants			
	Distance			
	0 cm	SE	10 cm	SE
6-methyl-5-heptene-2-one	0.20	0.01	0.16	0.01
(Z)-3-hexenyl acetate	0.21	0.01	0.24	0.01
Hexahydrofarnesyl acetone	0.57	0.03	0.51	0.04

Compounds	Isolated Plants			
	Infestation			
	Infested	SE	Uninfested	SE
6-methyl-5-heptene-2-one	0.44	0.02	0.33	0.02
(Z)-3-hexenyl acetate	0.32	0.02	0.19	0.01
Hexahydrofarnesyl acetone	1.09	0.06	1.85	0.01

Compounds	Multiple Plants			
	Infestation			
	Infested	SE	Uninfested	SE
6-methyl-5-heptene-2-one	0.19	0.01	0.17	0.01
(Z)-3-hexenyl acetate	0.27	0.01	0.19	0.01
Hexahydrofarnesyl acetone	0.48	0.03	0.60	0.04

APPENDIX H

EFFECT OF EXPERIMENTAL PLANT SET, COLLECTION TIME, AND
COLLECTION POSITION ON WHEAT VOLATILE COLLECTION

Effect of experimental plant set, collection time, and collection position on the average amount (ng) of volatile compounds collected from isolated and multiple plant experiments.

Compounds	Isolated Plants			
	Plant Set			
	Set 1	SE	Set 2	SE
6-methyl-5-heptene-2-one	0.41	0.03	0.37	0.02
(Z)-3-hexenyl acetate	0.25	0.01	0.26	0.02
Hexahydrofarnesyl acetone	1.49	0.10	1.45	0.09

Compounds	Multiple Plants			
	Plant Set			
	Set 1	SE	Set 2	SE
6-methyl-5-heptene-2-one	0.17	0.01	0.19	0.01
(Z)-3-hexenyl acetate	0.23	0.01	0.22	0.01
Hexahydrofarnesyl acetone	0.55	0.04	0.53	0.03

Compounds	Isolated Plants							
	Collection Time							
	T1	SE	T2	SE	T3	SE	T4	SE
6-methyl-5-heptene-2-one	0.41	0.03	0.35	0.02	0.37	0.03	0.42	0.03
(Z)-3-hexenyl acetate	0.36	0.03	0.27	0.02	0.21	0.02	0.18	0.02
Hexahydrofarnesyl acetone	1.15	0.13	1.09	0.10	1.64	0.14	2.00	0.14

Compounds	Multiple Plants							
	Collection Time							
	T1	SE	T2	SE	T3	SE	T4	SE
6-methyl-5-heptene-2-one	0.23	0.01	0.20	0.01	0.16	0.01	0.14	0.01
(Z)-3-hexenyl acetate	0.30	0.02	0.26	0.02	0.20	0.02	0.14	0.01
Hexahydrofarnesyl acetone	0.33	0.03	0.40	0.03	0.63	0.05	0.81	0.06

Compounds	Multiple Plants			
	Collection Position			
	Outside	SE	Inside	SE
6-methyl-5-heptene-2-one	0.13	0.01	0.21	0.01
(Z)-3-hexenyl acetate	0.14	0.01	0.28	0.01
Hexahydrofarnesyl acetone	0.38	0.02	0.65	0.04