

MASS REARING OF *Bracon cephi* (Gahan) AND *B. lissogaster* Muesebeck
PARASITOIDS OF WHEAT STEM SAWFLY, *Cephus cinctus* Norton, AND
TEMPERATURE-INDUCED MORTALITY IN HOST IMMATURES

by

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ABSTRACT

Bracon cephi (Gahan) and *B. lissogaster* Muesebeck are host specific larval parasitoids of the wheat stem sawfly, *Cephus cinctus* Norton. A reliable source of these parasitoids is needed for inoculative releases into sawfly infested wheat fields in Montana. Large walk-in field screen cages were used to confine sawflies and parasitoids on wheat. Factors affecting the successful establishment of sawflies in wheat and subsequent attack by parasitoids were tested. Treatments consisted of cage modifications such as windows for enhancing the amount of light, food sources for parasitoids, increased light plus food sources, and a control. Methods for delivering adult sawflies into the cages were also investigated. The mass rearing cages with the windows had significantly greater sawfly infestation and parasitism when compared to the cages without windows. The sawfly infestation was low the first year, while the percent parasitism was quite high, indicating that the major impediment to mass rearing was obtaining greater sawfly infestation. We obtained higher amounts of infestation and parasitism for the second year when the method of introduction of *C. cinctus* adults into the rearing cage was changed, and this difference was more obvious than any treatment effects. However, the sex ratio of the mass reared parasitoids was male biased indicating the absence of pre-mated females in the cages. The low number of male parasitoids in the rearing cages probably influenced this outcome. Experiments were conducted to assess possible temperature-induced mortality of sawfly immatures. Lethal temperatures and times for predicting mortality were calculated for the overwintering larval and pupal stages at 2 to 5 hr time intervals using probit analysis. The result shows that for both stages, mortality increased with increasing temperatures, and for a fixed temperature, the LT_{50} was lower for longer time intervals. The temperature-induced mortality experiments in the laboratory helped us to assess the lethal temperatures that should be avoided inside the rearing cages to enhance survival of the parasitoids. The temperatures inside the cages were significantly lower than those occurring in the field. These results provide the basis for wheat stem sawfly parasitoid mass rearing in walk-in cages.

CHAPTER ONE

LITERATURE REVIEW

Introduction

The wheat stem sawfly (WSS), *Cephus cinctus* Norton (Hymenoptera: Cephidae) was described in 1872 by Norton from an adult collected in Colorado. This sawfly, which originally fed within the hollow stems of feral grasses like Smooth Brome, *Bromus inermis* Leyss., Western wheat grass, *Pascopyrum smithii* (Rydb.), and Great Basin Wild Rye, *Elymus cinereus* (Scribn. and Merr.), shifted to wheat in the late nineteenth century (Ainslie 1920). Riley and Marlatt (1891) predicted that “it may be expected at any time to abandon its natural food plant in favor of the small grains”. This became a reality in 1896, when the wheat stem sawfly was first reported feeding on wheat in Manitoba. The wheat stem sawfly (WSS) preferred grass hosts until 1907, when greater levels of infestation were first discovered in wheat. Today, WSS is the most important insect pest of wheat, *Triticum aestivum* L in the Northern Great Plains of the United States and southern regions of the Canadian prairies. Cultural pest management like deep fall plowing, crop rotations with oats, *Avena sativa* L., and late planting which encouraged the natural enemies may have helped to suppress the WSS populations during the early d (Holmes 1978). The rapid expansion of wheat acreage, introduction of summer-fallow practices, and strip crop-fallow crop rotations, may explain the greater levels of WSS infestation in recent yrs (Weiss and Morrill 1992). It cannot be controlled using chemical methods (Morrill 1995).

Distribution of the Wheat Stem Sawfly

Several surveys conducted since 1947 describe the distribution of WSS in North America. The states of North Dakota, South Dakota, Nebraska, Wyoming, Colorado, Utah, Montana, Kansas, Minnesota, and Iowa had WSS in either grasses or wheat. Montana and northwestern North Dakota in the United States, and western Manitoba, Saskatchewan, and eastern Alberta in Canada had the heaviest infestations. Specimens of WSS were also collected from Oregon, Washington, California, Idaho, Arizona, New Mexico, Illinois, Missouri, Nevada, Michigan, and Ohio (Anonymous 1955). The WSS can survive in a wide range of environmental conditions, but population density might be dependent on several factors (Weiss and Morrill 1992). The WSS is assumed to be native to North America, but recently a hypothesis was forwarded stating that a recent introduction originated in northeastern Asia (Ivie 2001).

Within Field Spatial Distribution

Factors other than food could influence the spatial distribution of the WSS because spatial aggregations of insects have been observed even in crop monocultures. (Feng and Nowierski 1992, Holland et al. 1999, Schotzko and Quisenberry 1999, Winder et al. 1999, Korie et al. 2000). WSS infestations in the field were reported to have a distinct “edge effects” where the wheat stems at the field edges bordering either the fallow wheat crop from the previous season or grassland had comparatively higher infestation levels. The percentage of infestation decreased gradually towards the center of

the wheat field (Pesho et al. 1971, Holmes 1982, Morrill et al. 2001, Runyon 2001, Sing 2002, Nansen et al. 2005).

WSS Damage

In Montana, WSS has been estimated to cause annual losses in wheat exceeding \$25 million in recent yrs (Blodgett and Lanier 1996, 1997). It is a pest of both winter and spring wheat and infestation levels as high as 100% have been recorded in some wheat fields (Morrill 1994). WSS affects the yield in wheat crops both physiologically and physically. Physiologically, it affects the wheat plant by causing weight reduction of the kernels (Holmes 1962) and lowering the protein content of the grain by 0.6 to 1.2 % (Holmes 1977), while the physical loss is due to unrecoverable, lodged heads at harvest.

Selection of plants for oviposition by WSS depends on the development stage and size of the wheat plants (Holmes 1977). The primary stem and head appear first, followed by one or more smaller secondary tillers. The early season WSS adults of the season oviposit in the primary stems while late ones prefer secondary stems. Oviposition preference is also likely for large stems, which have the potential for producing the heaviest heads (Morrill 1992). WSS prefers to infest the wheat plant when it reaches the Zadoks 32 stage, when the second node is first visible. Infestation can still occur after plant development passes Zadoks 45 or the boot stage, even though it occurs less frequently (Nelson et al. 1988).

When stems of similar diameters were compared, the yield reduction was found to be around 11 to 22 % due to reduction in the number and size of the kernels per head (Holmes 1977). WSS larvae feed on the parenchymous tissue inside the wheat stem and

this might disrupt the nutrient and water uptake of the plant by damaging the vascular bundles (Weiss and Morrill 1992). There is also some recent evidence showing that the physiological responses of wheat to WSS injury could be primarily dependent on environmental factors (Macedo et al. 2005).

Physical loss occurs when a WSS larva cuts a ring around the inside of the stem near the soil surface, weakening the stem. If this is followed by wind or precipitation, variable amounts of stem lodging occur, reducing harvest efficiency (Ainslie 1920, Munro 1947). Harvest efficiency is affected in several ways at greater levels of WSS infestation, including the loss of mature wheat because the combine cannot collect all lodged stems, slower operation of the combine resulting in greater fuel consumption, and potential damage to the combine when operated in uneven, rocky soils due to the lowering of the combine header to collect lodged wheat stems. There may also be increases in the costs for application of herbicides to destroy volunteer wheat in the fall and spring (Nansen et al. 2005). In early records, WSS adults attacked spring wheat and winter wheat avoided most of the damage, probably due to its early maturation (Davis 1955). In 1989, extensive damage was reported by Weiss and Morrill (1992) in winter wheat in central Montana, with heavy yield losses. WSS adult females had adapted to winter wheat by commencing oviposition a month earlier, so by the 1989 both spring and winter wheat were heavily infested (Morrill et al. 1994, Morrill and Kushnak 1996).

WSS Detection

Feeding by WSS larvae at the stem node interrupts the upward and downward transport of carbohydrates, resulting in dark areas visible on the stem exterior. Based on

this visual cue, sawfly infestation can be determined as early as the soft dough stage. But these dark areas may occur in response to plant pathogens, so this character should be used cautiously (Morrill et al. 1992). The presence of WSS adults in the field and the amount of cutting from the previous yr are other methods for predicting the level of infestation (McBride 1989). WSS adults are weak fliers, thus the heaviest infestations of the pest are concentrated at field margins (Holmes 1982). An accelerometer and clamp system can also be used to detect the feeding activities and movement of different size of WSS larvae inside the wheat stem (Mankin et al. 2004).

WSS Biology

Oviposition

WSS adults emerge in late May or early June from wheat stubble fields and nearby grasses, with emergence period lasting 3-4 weeks (Morrill and Kushnak 1999). The flight period for each adult lasts as many as 11 d, during which egg maturation, mating, and oviposition takes place. WSS oviposits heavily during the first 4 d of the flight period (Holmes 1982, Morrill et al. 1992). Females produce an average of 33 eggs and usually lay one egg per stem, although many females may lay eggs in the same stem (Holmes 1978). Females prefer to oviposit in an elongating (uppermost) internode (Holmes and Peterson 1960). Early in the oviposition period the females will oviposit in the longest stems, but later as stems attain their full length, the females will select less mature stems (Holmes 1978). The adults have a habit of clinging close to the wheat stems and resting with their head downwards, especially during windy periods (Criddle 1922).

Larva

Temperature and moisture are important factors influencing egg development. The eggs hatch in 6-7 d and the emerging larva begins to feed on parenchymal tissue within the stem. If more than one egg is laid per stem, only one larva survives cannibalism (Ainslie 1920). There are 4-5 instars depending on the wheat stem quality (Farstad 1940). The larvae normally move upward through the stem, and are found in the upper internodes from mid- to late-July. They move down towards the basal internode by late-July or early-August (Holmes 1978). Completion of larval development usually coincides with plant senescence. Visible, infrared light transmitted through the stem and positive geotaxis may stimulate the movement of the larva to the basal internode (Holmes 1975, 1978). The larval period lasts approximately 60 d and varies with temperature and degree of stem maturity. The size of the larvae depends on the quantity and quality of food consumed, (i.e. stem quality) (Ainslie 1920).

Diapausing Larva

To complete larval development, the larva prepares to enter diapause at the base of the stem. It girdles the stem with a V-shaped notch entirely around the interior of the stem, above or below soil level (Ainslie 1920, Runyon et al. 2002). This activity is initiated when the stem moisture decreases and the moisture content of the kernel reaches between 41 and 51% (Holmes 1978). The circular cutting does not sever the stem completely, but weakens it so that the upper portion breaks off completely as it sways in the wind. The remaining portion of the cut-stem in the ground is called a wheat 'stub', coined by Wendell Morrill, which serves as an overwintering chamber for the larva.

Below this notch, the larva plugs the stub with frass. Absorption and retention of moisture by the frass plug for long periods could be detrimental to the diapausing larva (Ainslie 1920).

Later in the summer, the larva spins a transparent tube of filmy, silken tissue called the hibernaculum (Farstad et al. 1949) which is closed at both ends and free from the sides of the chamber, which remains intact until adult emergence. During the winter, the larva is in an obligatory diapause (Ainslie 1920). Obligatory diapause can be completed only by exposure to 10 °C or lower for at least 90 d in an environment containing about 12 to 15% moisture (Holmes 1978). The ending of the obligatory diapause of WSS larvae involves the completion of two steps. The first step is completed when the diapausing larva is exposed to sufficiently low temperatures for an adequate interval and the second step is completed when they are exposed to increasing temperatures required to metamorphose. If only the first stage in development is complete, the insect can be returned to diapause by either high temperature or lack of moisture or both, which could result in a two-yr life cycle (Salt 1947).

Pre-pupa and Pupa

In spring, after exposure to warming conditions linked to increasing photoperiod, the WSS larva breaks diapause to become active until pupation (Ainslie 1920). In early May, the larva begins to enter the pre-pupal stage, and the first pupae form in late May. The duration of development from post-diapause larva through the adult is 20 to 30 d at 25 °C (Holmes 1978). If the larva is exposed to 35 °C or continuous light, it will reenter diapause and require another 90 d at 10 °C. The following May, the larva pupates and the

adult chews an exit hole through the plug during late May or early June. Emergence is facilitated when the frass plug in the cut stem is moistened by precipitation (Holmes 1978).

Sex Ratio

WSS is haplodiploid, so the male is haploid (9 chromosomes) and the female is diploid (18 chromosomes). Unmated females produce fertile eggs, which give rise to male offspring. The mated female WSS can perform selective egg fertilization at the time of oviposition and this determines the sex of the progeny (Flanders 1946, Cook 1993). A typical population consists of approximately equal numbers of males and females (Farstad et al. 1949, McGinnis 1950). The adult males emerge before the females, ensuring that the earliest emerging females will be mated. As emergence progresses, the number of male sawflies decreases and the proportion of unmated females increases. This might result in male-biased populations if the late emerging females are unable to mate (Jacobson and Farstad 1952) or if there is deficiency of available sperm later in the season (Farstad et al. 1949, Jacobson and Farstad 1952).

Oviposition first occurs in the field margin next to the source of infestation and progresses towards the center of the field. Because of this, the male-to-female sex ratio is lower towards the field margins than in the field center (Holmes 1978). When field collection is performed using sweep nets to determine the sex ratio, the population might appear to be female biased because male WSS emerge first and die quickly (Jacobson and Farstad 1952, Holmes and Peterson 1963).

The sex ratio and fitness of WSS also could be influenced by factors like the

quality of its host plant (Mopper and Whitham 1992). WSS prefers stems with large diameters. The sex ratios are male-biased in small stems and female biased in larger stems. It was also reported that the adult size of female WSS, as well as their fecundity and longevity also increase with the width of the wheat stem (Morrill et al. 2000). This relationship between the quality of wheat stem and fitness was not seen in male WSS (Morrill and Weaver 2000).

Population Dynamics of WSS in Wheat

Mortality in Stubs

Mortality of WSS larvae was reported to range from 8 to 21%, and was calculated by enumerating the cut stubs from the previous yr and the number of adults that emerged from them (Holmes 1982). Low temperature can be an important mortality factor because larvae freeze at temperatures below -10°C and may also be killed by desiccation in combination with extremes of temperature (Salt 1946, 1961). Exposure of the pre-pupal and pupal stage of WSS to a temperature of 35°C for short periods or to continuous light suppresses post diapause development and causes re-entry into diapause. This may result in malformed pupae and adults (Villacorta et al. 1971). Excessive moisture could also result in higher larval mortality (Criddle 1921). Larval mortality was seen before reaching super-cooled (-24°C) temperatures (Morrill 1993). Exposure of WSS larvae and pupae to greater temperatures than 35°C should be studied further to verify its role as a mortality factor.

Factors Affecting Oviposition

Oviposition is reported to typically occur between 9 AM and 6 PM when the air temperature is between 20 and 31 °C, and the wind velocity near the soil surface is below 8 km hr⁻¹. At lower temperatures, winds of over 5 km hr⁻¹ prevented oviposition, while at temperatures higher than 31 °C, oviposition continued until the winds exceeded 8 km hr⁻¹ (Holmes 1982). Low temperature and heavy wind during the flight period delays infestation by WSS, but does not reduce the overall level of infestation. Fecundity is influenced by the wheat variety in which the adult developed, with the females that developed in susceptible hollow stem varieties having greater fecundity. The amount of precipitation during the oviposition period is also important as this softens the wheat stem for oviposition (Farstad 1945). Environmental conditions such as the availability of soil moisture also influences stem thickness, which in turn affects sawfly larval development and fitness (Holmes and Peterson 1962).

Larval Mortality

If the larvae cut 7-9% of the wheat in one yr, the adults emerging from these stubs may infest 70-80% of the stems of the adjacent wheat crop during the following yr (Holmes 1982). Cannibalism is observed in WSS larvae and this allows only one larva to survive in each stem. This limits population size slightly because different females can lay eggs in the same wheat stem (Holmes 1982). *Bracon cephi* is an important larval parasitoid of WSS that significantly reduces populations in the field (Holmes 1963). Host plant resistance is imparted by the solidness of the lower three nodes and is an important factor that regulates WSS populations (Holmes 1982). Mortality varies in both

susceptible and resistant varieties under varying weather conditions (Holmes 1982). The larva has an obligatory diapause after which the insect needs an adequate amount of moisture to complete development. Prolonged desiccation of the larvae at low temperatures post diapause results in greater mortality (Church 1955). Adult emergence was found to cease at temperatures below 10 °C, when most of the larvae were in quiescence, while at 35 °C they returned to diapause. WSS post diapause development time was shorter at temperatures of 20 °C and above, while it increased greatly at 15 °C. Temperature and relative humidity did not influence the weight or sex ratio of the emerging adults. The most suitable temperature for larval to adult development was found to occur between 20 and 25 °C at a relative humidity of 60 to 75% (Perez-Mendoza et al. 2006). Further studies should be conducted regarding the mortality of different stages of WSS at higher temperatures, for the better knowledge of its population dynamics.

WSS Management

Cultural Methods

Criddle (1911, 1913, 1915, 1922) recommended deep moldboard plowing, early mowing, reduced mowing in brome grasses to facilitate parasitoid emergence, planting trap crops, and early harvesting to manage WSS. These methods were followed by the farmers for some time with moderate results, but were not economical due to yield reductions associated with these methods and the adoption of improved mechanization (Weiss and Morrill 1992). WSS larval mortality of 90 - 93% was observed in stubble exposed on the soil surface by tillage. However, tillage is not recommended because of

water retention and soil erosion issues (Morrill 1993). Burning of wheat stubble in the field does not significantly reduce the number of diapausing larvae because many stubs are found below the soil surface. But this might kill the overwintering parasitoid cocoons in the wheat residue, and in addition, it creates an increased risk of soil erosion (Ainslie 1929). Delayed planting is considered another effective method to reduce WSS damage, but it also results in lower crop yield in wheat due to a less efficient use of soil moisture (Jacobson and Farstad 1952, Morrill and Kushnak 1999). WSS damage could also be reduced by conserving greater numbers of parasitoids (Runyon 2002, Meers 2005). This can be done by harvesting the wheat plant at a height of one-third of the wheat plant or greater, where 80% of the overwintering parasitoid cocoons occur in spring wheat (Meers 2005). There is no significant reduction of infestation in fields bordering heavily tilled fallow fields due to the ability of the adult WSS to dig through the soil during emergence. The parasitoids of WSS that overwinter above the ground level are affected by heavy tillage and are best conserved if the field borders contain untilled wheat residue (Runyon 2002).

Chemical Control

Experiments were conducted to evaluate the performance of different insecticides against WSS. None of the insecticides tested produced satisfactory results (Munro et al. 1949). Mating and oviposition of the wheat stem sawfly can be completed in a d, so only highly toxic, fast-acting contact insecticides can regulate the adult population and oviposition in the field. Ten contact insecticides evaluated for this purpose did not give good results (Holmes and Hurtig 1952). Seed treatment insecticides were also evaluated,

some of which gave good control but resulted in significantly decreased plant stands (Wallace 1962). Heptachlor was only effective when infestation levels were low (Holmes and Peterson 1963). The effect of insecticides on the success of the overwintering WSS or parasitoids was not significant (Morrill and Parker 1982).

Resistant Wheat Varieties

Use of solid-stemmed wheat varieties is also considered to be an important method of reducing the crop losses caused by WSS. The first resistant wheat variety was 'Rescue' and after its release the infestation rate decreased by 59% (Platt et al. 1948). Research conducted by Morrill et al. (1992) suggested that WSS infestation in solid stem varieties ranged from 16 – 47%, questioning the value of increased pith for suitable pest management. But further studies showed that the solid-stem varieties further increase the WSS mortality by preventing the larva from reaching the basal portions of the stem where it overwinters. Stem solidness did not affect parasitism rates, which makes it a suitable tool in an integrated pest management strategy (Morrill et al. 1994).

Biological Control

WSS spends three life stages (egg, larva, and pupa) inside the wheat stem and currently cannot be efficiently controlled by chemical methods. WSS populations in native and feral grasses are frequently associated with abundant natural enemies. Nine parasitoid species are reported to occur on WSS in native and feral grasses (Holmes 1953, Nelson 1953, Davis et al. 1955) and seven are native to the United States - *Bracon cephi* (Gahan) and *B. lissogaster* Muesebeck (Hymenoptera: Braconidae), *Eupelmella vesicularis* Retzius (Hymenoptera: Eupelmidae), *Eupelmus allynii* French (Hymenoptera:

Eucharitidae), *Eurytoma atripes* Gahan (Hymenoptera: Eurytomidae), *Pleurotropis utahensis* Crawford (Hymenoptera: Chalcididae), and *Scambus detritus* (Holmgren) (Hymenoptera: Ichneumonidae) (Davis et al. 1955). These parasitoids were successful on sawfly populations in the native and feral grasses, but when WSS invaded wheat fields in response to cultivation, it succeeded in escaping these parasitoids and rapidly evolved as a major pest of wheat (Criddle 1924). Early in the cultivation of wheat, levels of parasitism of WSS reached 100% in grasses while they were less than 2% in adjacent wheat (Criddle 1923, Neilson 1949).

Two parasitoids, *Collyria calcitrator* (Gravenhorst) (Hymenoptera: Ichneumonidae) and *B. terebrella* Wesmael (Hymenoptera: Braconidae), from the European WSS, *C. pygmaeus* (L.), were imported from Europe to be released against WSS. These agents were unable to establish for long term biological control of the wheat stem sawfly (Davis *et al.* 1955, Turnbull and Chant 1961). It was postulated that these parasitoids were specific to the European WSS host and therefore failed to establish (Smith 1961). Releases of *Heterosphilus cephi* Rohwer and *Pediobius nigratarsis* (Thompson) over a 9-yr period were also unsuccessful (Clausen 1978).

In wheat, WSS larvae are parasitized by only two of the 9 native parasitoid species: *B. cephi* and *B. lissogaster*. Both of these larval parasitoids are protelean, host-specific idiobiont ectoparasitoids with similar life histories and biology (Somsen and Luginbill 1956). Of the two parasitoids, *B. cephi* is predominant and has been reported to efficiently suppress WSS populations in Canada and Montana (Nelson and Farstad 1953, Holmes *et al.* 1963, Morrill *et al.* 1994, Ainslie 1929). In central Montana, the two braconid parasitoid species were reported to suppress 98% of the WSS larvae available in

the field (Morrill et al. 1998). A new predator of WSS immatures, *Phyllobaenus dubius* (Wolcott) (Coleoptera: Cleridae), was recorded in 2001 (Morrill et al.).

Biology of *B. cephi*

Egg

B. cephi is an idiobiont host specific larval parasitoid that paralyzes the host during oviposition (Nelson and Farstad 1953, Wharton 1993). There is a pre-oviposition period of three weeks in the first generation during which the eggs mature. The process is gradual and the eggs generally become mature in batches of 6, 8, or 10. The females lay 2 to 4 eggs daily and the oviposition event lasts for an hr. The eggs are 0.9 mm in length and are laid on or near the host. This parasitoid species lays one egg per host and the egg has an incubation period of 1 to 2 d (Nelson and Farstad 1953).

Larva

There are 5 instars and all have well-developed, sclerotized mouthparts. The first instar has a head that is proportionately larger than its body and the later instars can be identified by the presence of white irregular urate crystals close to the integument (Nelson and Farstad 1953, Cherian and Narayanaswami, 1944). The fully-developed larva has 13 body segments and no appendages are conspicuous for any of the instars, except for the non-segmented antennae (Nelson and Farstad 1953). The newly-hatched larva, upon locating its host, attaches to the host using the mandibles and starts feeding on the surface of the host through minute lacerations (Somsen and Luginbill 1956). After feeding, only the integument of the host remains. A fully grown larva spins a

cylindrical cocoon inside the stem, which is held firmly in the stem by a disc plate at each end. The larval stage lasts for 10 d. The winter is passed as a mature larva in this cocoon. The cocoons for the first generation are usually flimsy and are attached to one side of the stem. The larva inside the cocoon is smaller and paler than the last feeding instar. The developing prepupae are flattened dorsoventrally and develop prominent hypopleural swellings. The urate crystals become less conspicuous at this stage (Nelson and Farstad 1953).

Pre-pupa and Pupa

Immediately before the prepupal stage, the larva becomes highly active, and the subsequent prepupal stage last 2 d. This stage can be recognized by the constriction that develops behind the third thoracic segment. Pupation takes place within the cocoon and the pupa is not capable of movement. The duration of this stage is approximately 6 d under laboratory conditions. The pupa is white and delicate, with the eyes generally turning pink during this stage (Nelson and Farstad 1953).

Adult

The adult parasitoid emerges by chewing a circular hole through the cocoon and the stem. Occasionally the adults are trapped inside the stem when they exit the cocoon into the stem lumen. Under laboratory conditions, adult males were found to live for 10 to 14 d and adult females for approximately 4 wk (Nelson and Farstad 1953). But under field conditions, males emerged by the first week of June and were present until the end of July, while females started emerging in the last week of June and were still present when the second-generation females started to appear in the middle of August (Nelson

and Farstad 1953). In Canada, the second-generation females were found in the field until the end of September and in some cases survival is limited by low temperature conditions (Nelson and Farstad 1953). The life history of *B. cephi* is illustrated in Figure 1.

Figure 1. Life history of *B. cephi* in Western Canada (from Nelson and Farstad 1953).

STAGES		MAR	APR.	MAY	JUNE	JULY	AUG.	SEPT	OCT.
First Generation	Egg								
	Larva								
	Pupa								
	Adult								
Second Generation	Pupa								
	Adult								
	Egg								
	Larva								

Field Behavior

B. cephi adults feed on droplets of moisture on the leaves and on nectar from other sources and unlike WSS adults, these parasitoids can fly for a considerable length of time, and cover greater distances (Nelson and Farstad 1953). When the wind velocity increases, flight activity ceases and they move near the ground, clinging under the leaves. The adults are positively phototactic, and decreased activity is observed in cool weather. Continuous hot, dry weather for many d can increase adult mortality (Nelson and Farstad 1953).

The adult female parasitoids walk up and down the stem several times, stopping periodically to straddle the stem with their antennae, presumably to detect WSS larvae inside the stem as oviposition targets. After determining the general position of a suitable host in the stem, the female pauses until the WSS larva is suitably positioned and then inserts the ovipositor to sting the host. The egg is deposited on or near the paralyzed WSS host. The rate of parasitism increases after rainfall, which might be due to increased adult activity and the softening of the wheat stems enabling easier insertion of the ovipositor (Clausen 1940, Nelson and Farstad 1953).

Biology of *B. lissogaster*

This parasitoid was first described by Muesebeck (1953) as a new species and is reported from some wheat growing regions in Montana (Morrill et al. 1994, 1998; Morrill 1997). The biology of *B. lissogaster* is similar to that of *B. cephi*, except that the female lays 1- 4 eggs per paralyzed WSS larva. Therefore, it is a solitary or frequently gregarious ectoparasitoid and sometimes 3 to 4 adult parasitoids can be found from a single WSS host. Because of this gregarious parasitism, the size of the larva and adult is typically smaller than those which feed solitarily. Cannibalism is not seen among the parasitoid larva. Progeny from unmated females are males, while those of the mated females are both males and females, usually in equal numbers. The pupa is exarate and similar to that of *B. cephi*. The cocoons are loosely woven in the first generation, while they are tightly woven in the second generation. Under laboratory conditions, the longevity of the adults varies from 3 to 19 d with the availability of moisture and sugar (Somsen and Luginbill 1956).

Identifying Adult *B. cephi* and *B. lissogaster*

The adults of these two species can be readily distinguished (Runyon et al. 2001). This is an important aspect of explaining the population dynamics of WSS populations in wheat growing areas; especially to ascertain which particular species is dominant in areas with locally abundant parasitoids. There are four morphological characters (Runyon et al. 2001) that can be used for the separation of adults of the two species (Table 1). Body size is not a reliable index for separation of the species. Even though the body color is variable, it can be used with caution for the initial separation of the two species. *B. cephi* males and females usually have yellowish orange bodies, but sometimes the smaller specimens have a dark head and thorax. *B. lissogaster* males typically have a dark head and thorax with a yellowish orange metasoma, and *B. lissogaster* females have a black head with an orange body.

Table 1. Morphological characters used to separate *B. cephi* and *B. lissogaster* (from Runyon et al. 2001).

Character	<i>B. cephi</i>	<i>B. lissogaster</i>
Surface texture of metasoma	Finely granular	Smooth and polished
First metasomal suture	Crenulate	Fine, margined by a weakly sinuate ridge
Origin of vein 'r'	Near middle of stigma	Before middle of stigma
Second sub marginal cell	Long (ratio of 3RSa to r between 2.0 and 3.0)	Short (ratio of 3RSa to r between 1.0 and 2.0)

Variation in Parasitoid Effectiveness

If the second generation of the parasitoid species emerges late, they might not be able to complete their life cycle. Even though parasitism in postharvest wheat stubble is seen, it is not common and late emergence of this second generation of parasitoids could lead to lower parasitoid numbers in the following year (Holmes 1963). Increasing host numbers in the field are usually accompanied by low levels of parasitism and can be attributed to several factors. A parasitized WSS larva is more likely to be cannibalized if there are multiple larvae inside the wheat stem. This behavior of WSS larvae suppresses the potential population density of these parasitoids at higher densities. Another possible explanation of reduced parasitism at increased host density is that the parasitoid might not be able to locate the host due to the increased concentration of the chemical cues used in host-seeking, as has been reported in other systems (Flinn and Hagstrum 2002, Smith 2004, Smith and Press 1992).

If WSS larva develops inside the stem for prolonged periods, a higher percentage of parasitism is seen. If WSS larva cuts the stem later, then this allows for a successful second generation of the parasitoids. The ripening of the wheat governs the cutting by WSS (Holmes, 1963). The amount of soil moisture, the temperature experienced during crop development, and the date of seeding all influence the date of crop ripening and hence the parasitism (Holmes, 1963). Hyperparasitoids like *Eupelmella vesicularis* Retzius, and *Eurytoma attripes* Gahan have been reported from WSS parasitoids. These parasitoids reportedly attack both *B. cephi* and WSS and influence the effectiveness of WSS biological control (Nelson 1953).

For several yrs, efforts have been underway to redistribute overwintered *B. cephi* and *B. lissogaster* to locations experiencing losses due to WSS, using the methodology described in Morrill et al. (1998). In 2003, releases were made on seven farms throughout Montana and parasitoid establishment was limited. Samples collected at the early harvest in 2003 contained varying, but low numbers of atypically dead larger WSS larvae in the stems, with no evidence of disease or parasitoids (Weaver and Morrill, unpublished data). There was more than a week when the temperature was $> 35^{\circ}\text{C}$ at all release locations during the growing season, and from 1 to 6 d $> 38^{\circ}\text{C}$, depending on the location. Parasitoids were again released in 2005 and establishment was greater than in 2003. There was no evidence of atypical mortality in large WSS larvae in 2005, and no location reported temperatures $> 35^{\circ}\text{C}$ during the growing season. These observations suggest that parasitized, paralyzed larvae might be more vulnerable to high temperatures than unparasitized larvae, especially if healthy larvae can behaviorally avoid extreme heat.

The foraging behaviors of adult parasitoids are thought to be influenced by physiological state, which is dependent on mating status and food (Lewis et al. 1990). Studies on *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) parasitizing *Helicoverpa zea* (Boddie) in cotton, *Gossypium hirsutum* L., showed that the presence of nectar/sugar sources influenced the rate of parasitism and the retention of parasitoids in a particular patch. The study also showed that there was an innate attraction to extrafloral nectar sources, whereas through learning, the sugar source was also found to be equally attractive to the parasitoids. Host search efficiency was not influenced by the availability of food sources for *M. croceipes* parasitizing *H. zea* in cotton (Stapel 1997). An

experiment with *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), indicated that flowering plants used as nectar sources enhanced its survival (Gourdine et al. 2005). However, the rate of parasitism was not influenced by the availability of nectar sources. Another experiment with *Phanerotoma franklini* Gahan (Hymenoptera: Braconidae), a parasitoid of cranberry fruitworm, *Acrobasis vaccinii* Riley (Lepidoptera: Pyralidae) showed that presence of food sources increased longevity (Sisterson et al. 2002). However, nectar feeding did not influence the attack rate of the parasitoids or the egg maturation, while lack of feeding increased the mortality rate and decreased the host foraging ability of the surviving wasps (Sisterson et al. 2002). More studies are needed in this area to determine if flowering plants and other food sources could influence the parasitism of the wheat stem sawfly larvae.

Occasionally variation is seen among isolated populations of parasitoids and these biologically different groups are called “geographic races” (Clarke and Walter 1995) or “adapted strains” (Morrill 1998). Variation is seen in the levels of parasitism in different wheat growing regions in Montana. Some populations are more effective in parasitizing sawfly larvae than others. The level of parasitism was increased when individuals from an “adapted strain” were released in regions of low parasitism levels (Morrill 1998). Intraspecific variation is also seen in the foraging behavior of parasitoids and this could be attributed to factors such as genotypic differences, phenotypic plasticity in which the individual modifies its behavior through learning, and to the physiological state of the parasitoid (Lewis et al. 1990).

Objectives of This Project

Overall Objectives

The overall objective is to successfully mass rear *B. cephi* and *B. lissogaster*, as redistributable parasitoids of WSS, in large cages in wheat fields. The ability to do this would ensure a sufficient supply of parasitoids for release in wheat growing areas where there are high levels of WSS infestation and low parasitoid populations. Large populations of the parasitoids *B. cephi* and *B. lissogaster* can reduce WSS populations satisfactorily, but we do not typically see large numbers of parasitoids in fields experiencing heavy losses due to WSS.

A second objective is to assess possible host mortality that might occur at high temperatures, as a potential limitation to the successful mass rearing of parasitoids, using overwintered immature stages of WSS as a model system. These data can be used to determine potential limitations on parasitoid mass rearing, but do not address whether mortality might occur at differing temperatures for the developing parasitoids or actively-feeding larvae. The logistic complexity of assessing temperature-induced mortality in developing parasitoids or actively-feeding larvae precludes direct experimentation.

Hypotheses for the Experiments

1. The levels of WSS infestation and parasitism of WSS larvae are dependent on light intensity, presence of nectar and sugar in aqueous solution as food sources, or a combination of both.
2. The level of infestation, and thus host availability for the parasitoids is dependent on the method used to deliver WSS inside the rearing cages.

3. High temperatures that cause mortality in immature WSS overlap with those that could be experienced in wheat plants in mass rearing cages.

Null Hypotheses

1. There is no difference in the level of WSS infestation or parasitism of WSS larvae under different levels of light intensity or with available nectar and sugar in aqueous solution as a food source.
2. The method of introduction of WSS inside the rearing cages has no effect on the level of infestation, and thus does not impact host availability for the parasitoids.
3. High temperatures that cause mortality in wheat stem sawfly immatures do not overlap with those experienced in growing wheat plants.

Objective 1

The first specific objective is to determine if variable visible and ultraviolet (UV) light intensity, the availability of nectar and sugar solutions as food sources, or a combination of both would influence the levels of infestation and parasitism in the rearing cages. If any variation in infestation and parasitism levels is caused by these factors, it will indicate if they have major influences on the mass production of *B. cephi* and *B. lissogaster*.

Due to the high cost of rearing cages, the light intensity in the cages was increased by adding windows made of material that transmits high amounts of UV and visible light. The role of visible and UV light in infestation and parasitism needs to be determined, and

the effectiveness of the other factors tested in the field rearing cages also will be assessed under greenhouse conditions.

Objective 2

The second specific objective will determine if the method adopted for introducing WSS adults into the rearing cages in the field influences the infestation level in wheat. The amount of parasitism depends on the host availability in the rearing cages, so this will determine if the difference in the introduction method is influencing the mass production of parasitoids more than the availability of other factors like light, and food sources for the larval parasitoids.

Objective 3

The third specific objective is to determine mortality of the wheat sawfly at high temperatures. This objective will require measurement of temperatures occurring experimentally and in the field, as well the assessment of temperature-induced mortality in larvae and pupae. Specific objectives are:

- a) The measurement of temperature variation within the wheat canopy, as well as inside and outside the wheat stem at different heights within the growing wheat canopy.
- b) To determine the lethal temperature for 50% of the test population (LT_{e50}) and lethal time for 50% of the test population (LT_{50}) of the overwintering larvae and the pupae of WSS, as a model for potential mortality in actively feeding larvae.

It is not possible to reliably parasitize WSS larvae under laboratory conditions.

Therefore, paralyzed larvae and developing parasitoids are not available to study

thermally-induced mortality, so an experiment was undertaken to assess mortality in actively-feeding larvae. These data do not represent a realistic assessment of mortality due to complications imposed by the ability of the unparasitized larvae to thermally regulate within the stem by moving along its length. It is possible to dissect actively-feeding larvae and constrain them for exposure to a constant high temperature. However, the dissimilarity between immobilizing against an innate tendency to move to remain cool and potential changes in thermal tolerance due to a paralytic, immobilizing toxin are significant. For these reasons and for convenience, the naturally immobile immatures that have been overwintered are more suitable for experimentation than actively feeding larvae that have been removed from the stem.

Study of temperature-induced mortality of overwintered, immature WSS larvae is important in population dynamics. Significant mortality of WSS is occasionally seen in stubs in field populations. These experiments will determine whether high temperatures could contribute to the observed mortality in the field.

CHAPTER TWO

MATERIALS AND METHODS

Mass Rearing of *B. cephi* and *B. lissogaster* (Objective 1)

WSS maybe naturally and effectively controlled in many locations by its two major parasitoids, *B. cephi* and *B. lissogaster*. These experiments were conducted to determine if large field cages could be used as nurseries for parasitoids needed for inoculative releases into fields.

Infestation and Parasitism in Greenhouse Cages

A preliminary experiment was conducted in the greenhouse before the field experiments' to quantify the levels of ultraviolet and visible light, as well as the potential of nectar sources for enhanced rearing of the braconid parasitoids of the wheat stem sawfly. The possibility of rearing parasitoids in the greenhouse was also tested, despite earlier evidence that this was not feasible. Based on this information, optimal materials for ultraviolet and visible light transmission were selected prior to the experiment.

Selection of UV Transmitting Material: The transmission of visible and ultraviolet radiation through different materials was evaluated. Based on manufacturer specified data, nine different materials along with the mass rearing cage mesh were selected from a large pool and tested for transmission properties. The nine materials tested for ultraviolet and visible light transmission were: 1) a corrugated polycarbonate sheet (Gothic Arch Greenhouses, Mobile, AL); 2) a GT 6 mm infrared retention/ anti-condensate (Gothic

Arch Greenhouses, Mobile, AL); 3) a verolite twin wall polycarbonate (Gothic Arch Greenhouses, Mobile, AL); 4) Lexan Thermoclear® TC2 112 polycarbonate (GE Plastics, Dix Hills, NY), 5) Barlo XT-1 (polymethyl methacrylate, Derrylin, Northern Ireland); 6) Barlo XT-2 (polymethyl methacrylate, Derrylin, Northern Ireland); 7) Barlo XT-3 (polymethyl methacrylate, Derrylin, Northern Ireland); 8) Acrylite® OP4 acrylic sheets (Cryo Industries, Chicago, IL); and finally, the existing mesh covering the mass rearing cages, 9) amber lumite 7250Q screen of with 530 µm mesh openings (BioQuip Products, Rancho Dominguez, CA). A 16 x 16 x 16 cm box was lined with black cloth to minimize reflection. A 10 x 10 cm window was cut in the top of the box where the different transparent materials to be tested were placed for passing UV and visible light spectrum. The source of light, Paralite® 250W R40 flood bulb (Full Spectrum Solutions Inc, Jackson, MI) was placed directly above the window at a height of 5 cm. A Model LI-1400 datalogger light sensor for measuring light in $W m^{-2}$ (LI-COR, Inc., Lincoln, Nebraska) and a Model 40736A heavy duty ultraviolet light meter for measuring UV in $mW cm^{-2}$ (Extech Instruments, Waltham, MA) were placed directly under the source at a distance of 21 cm. The radiation measurements were recorded and the percentage transmission was calculated in comparison to light passing through air only. To support these laboratory calculations, a similar experiment was run in sunlight and the percentage of ultraviolet and visible light transmitted was recorded. Based on these results, the most suitable ultraviolet and visible light transmitting material was selected for the building windows for field and greenhouse cages.

Rearing in Greenhouse Cages: The experiment was conducted in a greenhouse using cages of the dimension 46 x 46 x 91 cm. In the greenhouse, plants were grown at 22 °C during photophase and 20 °C during scotophase. The soil used for raising the ‘McNeal’ spring wheat plants consisted of 50:50 mix of Plant Growth Center soil mix (equal parts of Bozeman silt loam soil: washed concrete sand: Canadian sphagnum peat moss) and Sunshine Mix #1 (Canadian sphagnum peat moss, perlite, vermiculite, starter nutrient charge, wetting agent, and dolomite lime). The pH of the soil was approximately 5.5-6. The fertilizer mixture used was Peters 20:20:20 (general) at 200 ppm Nitrogen per week. This fertilizer was applied at 100 ppm of Nitrogen twice weekly, on Tuesdays and Fridays. The plants were not fertilized until they reached the 3-leaf stage which was about 20 d. The plants were irrigated daily and the greenhouse photoperiod was 15L: 9D. Ambient lighting in the greenhouse is supplemented by GE Multi-Vapor MVR1000/C/U lights (GE Lighting, General Electric Company, Cleveland, OH), which do not generate the ultraviolet portion of the spectrum, and the glaze on greenhouse plastic does not allow the ultraviolet portion of the spectrum to penetrate. This glaze protects the greenhouse plastics from rapid degradation by ultraviolet light.

The cages used were of three types: (1) all sides were covered with fine mesh-screen (530 µm) as the low light treatment, (2) two parallel sides were covered with fine mesh-screen and the other two parallel sides with plexiglass which was the high visible light treatment, and (3) two parallel sides were covered with cage mesh screen and the other two sides were with transparent OP-4 acrylic material for optimal transmission of visible and ultraviolet radiation. All cages had fine mesh screen on the top and a wooden base for supporting the pots containing the wheat plants. Each cage had small doors to

facilitate introduction of the adult WSS and parasitoids. The cage design details are given in Table 2. ‘McNeal’ wheat plants were infested at the Zadoks 32 stage. Newly-emerged WSS were released into the cages in the numbers shown in Table 3. After 10 d, adult parasitoids were released into the cages.

Table 2. Cage components for each treatment used in the greenhouse rearing experiment (2004). ‘Mesh’ is Amber lumite screening (Type 7250Q) with 530 μm mesh openings, ‘OP4’ is OP4 Acrylite[®], which transmits both ultraviolet and visible light, and ‘Pg’ is plexiglass, which transmits visible light only.

Treatment	CAGE MATERIAL						Food source
	Side 1	Side2	Side3	Side4	Bottom	Top	
1	OP4	Mesh	OP4	Mesh	Wooden	OP4	Canola + honey solution
2	Pg	Mesh	Pg	Mesh	Wooden	Pg	Canola + honey solution
3	OP4	Mesh	OP4	Mesh	Wooden	OP4	None
4	Pg	Mesh	Pg	Mesh	Wooden	Pg	None
5	Mesh	Mesh	Mesh	Mesh	Wooden	Mesh	Canola + honey solution
6	Mesh	Mesh	Mesh	Mesh	Wooden	Mesh	None

These experiments were based on the assumptions that UV and visible light levels may influence WSS or parasitoid oviposition behavior and that food sources would increase the rate of parasitism. Certain cuticular hydrocarbons and diacetates of the WSS adult could undergo slow natural oxidation, possibly leading to the production of sex pheromones. The oxidation process might require the presence of many environmental

factors like ultraviolet and visible light (Bartelt et al. 2002), which explains their selection as treatment factors. The experiment was also conducted to determine if the laboratory studies would follow the same pattern as field studies.

Table 3. Treatments for the greenhouse cage rearing experiment with the number of WSS and *B. cephi* females released (2004).

Treatment	WSS females released	<i>B. cephi</i> females released
Ultraviolet and visible light plus a food source	9	6
Visible light plus a food source	9	6
Ultraviolet and visible light	9	6
Visible light	9	6
Food source	9	6
Control	9	6

Six treatments were used for the studies. Three wheat pots were placed inside each cage. A pot of flowering canola (*Brassica rapa* L.) and two vials of honey water mixture were used as food sources for the larval parasitoids. Ambient and supplemental greenhouse lighting with two additional supplemental bulbs (GE, 120W, Plant Gro N Show, Charles Walters, Sheridan, MT) were the visible light sources. A Paralite® 250W R40 flood bulb (Full Spectrum Solutions Inc, Jackson, MI) was used as the UV source.

In all the above treatments, nine females and seven male WSS adults were released. Six adult female and two adult male *B. cephi* were released into each of the six cages. The parasitoids were obtained from Ophiem, Montana on 30 July and introduced on 31 July, 2004. The wheat plants inside the cages were uprooted when all the

parasitoids were dead, four weeks after their release. Infestation and parasitism was determined through stem dissection using an X-ACTO® knife.

The experiment was based on the hypothesis that the greatest parasitism should occur inside the cage having all factors, supplemental visible and ultraviolet light, along with a nectar source for the parasitoids. Moderate parasitism should be present in cages with ultraviolet and visible light alone or the nectar source alone or light alone. The lowest parasitism should be in cages with none of the three factors.

The experiment was repeated in 2005 with a change in design based on the previous results. We used nine cages of the same dimensions 46 x 46 x 91 cm. There were three treatments with three replications each. The treatment details are given in Table 4. The nine cages were placed outside the greenhouse for maximum availability of visible and ultraviolet light. Three pots of ‘Reeder’ wheat plants were placed inside each cage and were infested with WSS at Zadoks 32 stage. Infestation was achieved by releasing seven female and three male WSS into each cage. After 10 d, four female and two male parasitoids of *B. cephi* were released into the cages. After a period of 4 wk, the plants were uprooted, labeled and dissected using an X-ACTO® knife. Variations in infestation and parasitism levels were recorded for the different treatments.

Table 4. Treatments for the greenhouse cage rearing experiment with the number of WSS and *B.cephii* females released (2005).

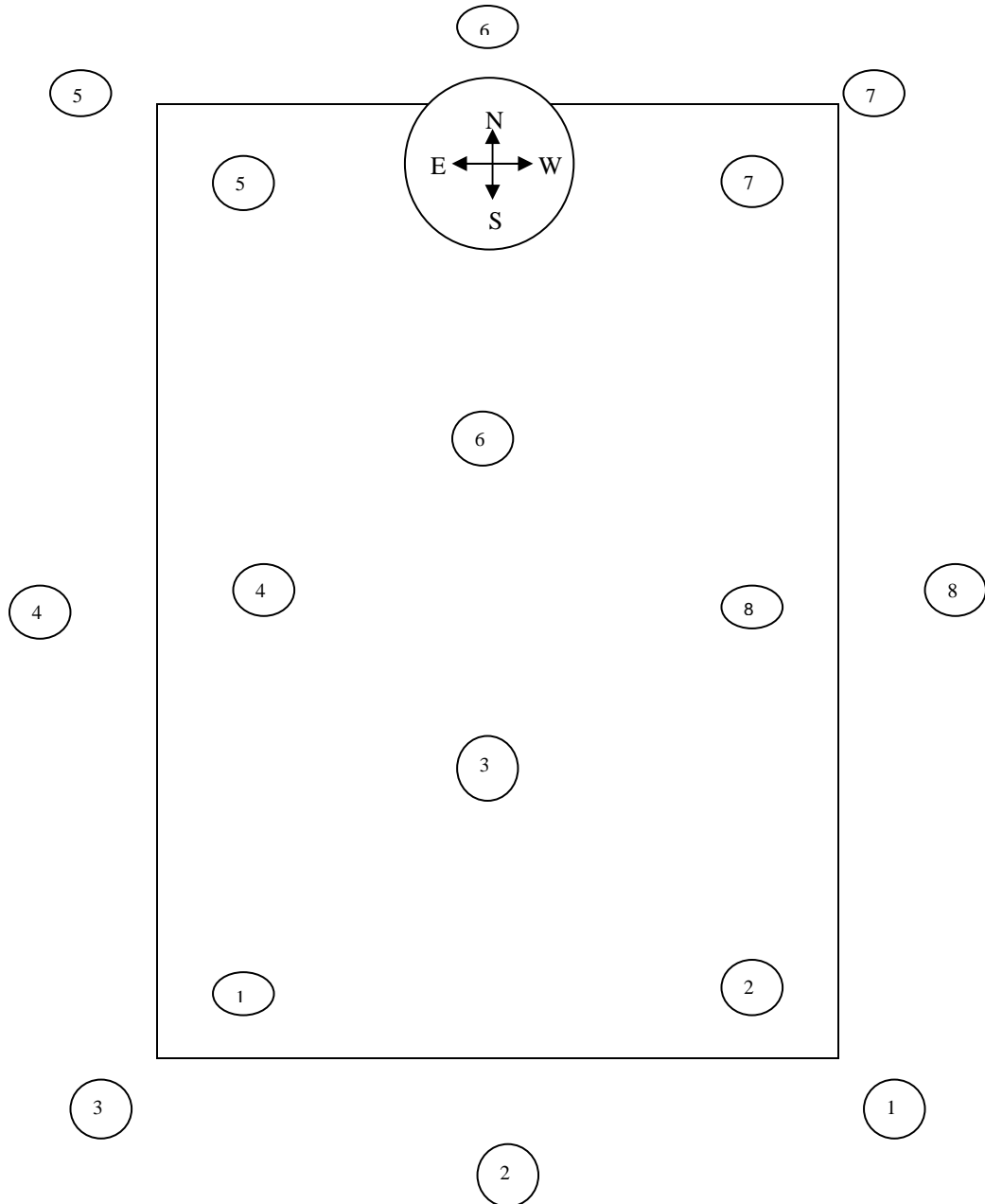
Treatment	WSS females released	<i>B. cephi</i> females released
Ultraviolet and visible light	7	4
Visible light	7	4
Control	7	4

Mass Rearing of Parasitoids in Field Cages

Visible and Ultraviolet Light and Temperature Variations (2004 - 05): At the location of the parasitoid rearing cages in the field at the MSU Post Farm, variation in temperature, visible and ultraviolet light intensity was measured at different positions inside and outside the cages. This would help to determine possible environmental changes in cages with or without windows, and could aid in the interpretation of the results. Five sets of observations were taken in a span of 3 d on 3, 4, and 6 Aug, 2004. All readings were taken approximately at 1 m from the soil surface and were above the wheat canopy. Eight readings were taken inside and outside the 12 cages at each strategically selected position (Figure 2).

Temperature readings were recorded using a non-contact infrared thermometer (Extech Instruments, Waltham, MA). A model LI-1400 datalogger light sensor was employed for measuring light in W m^{-2} (LI-COR Inc., Lincoln, Nebraska) and a model 40736A heavy duty ultraviolet light meter was used for measuring UV in mW cm^{-2} (Extech Instruments, Waltham, MA). Statistical analysis was performed using the ANOVA procedure of SAS (PROC GLM) to determine significant differences in temperature, light and ultraviolet measurements inside and outside the cages, between window and no window treatments, and between different positions inside the cages. Means and standard errors were calculated and the measurements were repeated in 2005.

Figure 2. Sampling points for temperature, visible, and ultraviolet light measurements taken inside and outside the mass rearing cages (2004-05).



Parasitoid Mass Rearing (2004): The WSS susceptible wheat variety ‘McNeal’ was planted in the field at the Montana State University, Bozeman, Post Farm, on 3 May, 2004. Twelve cages 3.60 x 1.75 x 1.68 m were made of iron frames and were covered with amber lumite screening (type7250Q) with 530 μm openings. This mesh screen was securely fastened to the cage frame using cable ties. Each screened cage was then anchored firmly to the ground using anchoring ropes, metal clamps, and iron posts. These additional attachments prevented wind damage to the cage. The bottoms of the cages were sealed with a volume of soil to prevent the escape of the parasitoids. There were 12 1.8-m rows of wheat planted east to west and the cages were oriented north to south along the long axis. There were four cage treatments based on the presence or absence of supplemental UV and visible light, food source for parasitoids, or a combination of both. Each treatment was replicated three times and the treatments were randomly assigned to the 12 cages. The layout of the cages in the field is illustrated in Figure 3.

Cages that incorporated windows used OP4 acrylic material that allowed increased transmission of ultraviolet and visible light. There were six 88.9 x 43 cm windows supported by wooden frames in each of these cages. An outline of the cage design is shown in Figure 4. Two flowering plant species, canola and yellow sweet clover (*Melilotus officinalis* (L.) Lam.) were used as supplemental nectar sources for the parasitoids in the appropriate treatments. These flowering species were raised in greenhouse and were transplanted into the field after flowering. The conditions and substrate used for raising the canola and sweet clover plants in the greenhouse were

Figure 3. Layout of the mass rearing cages at the Post Farm (2004-05).

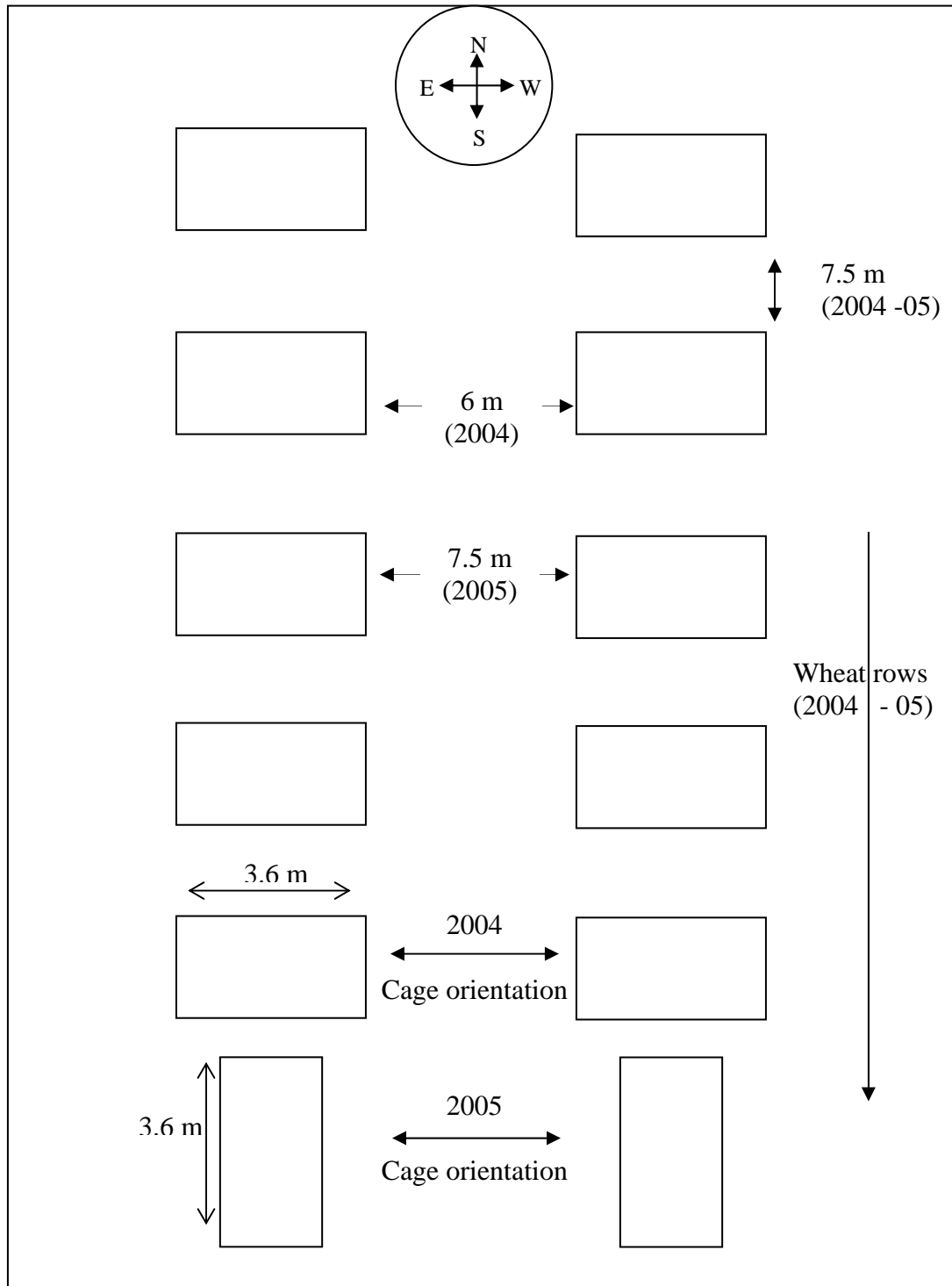


Figure 4: Diagram illustrating dimensions for the field rearing cages used at the MSU Post Farm (2004-05).

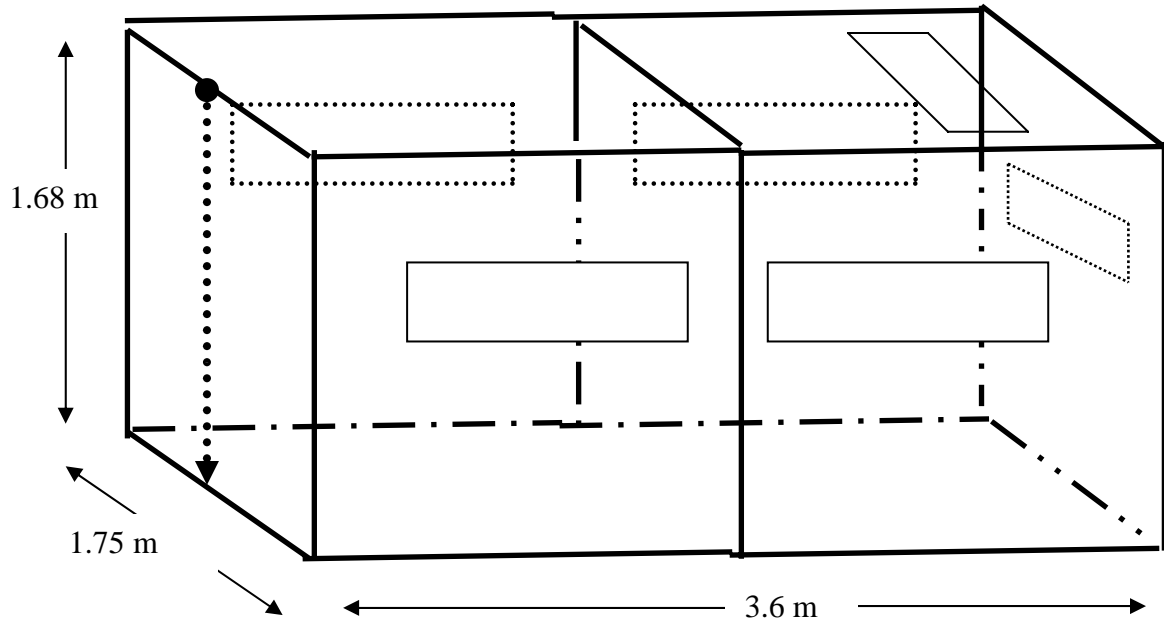
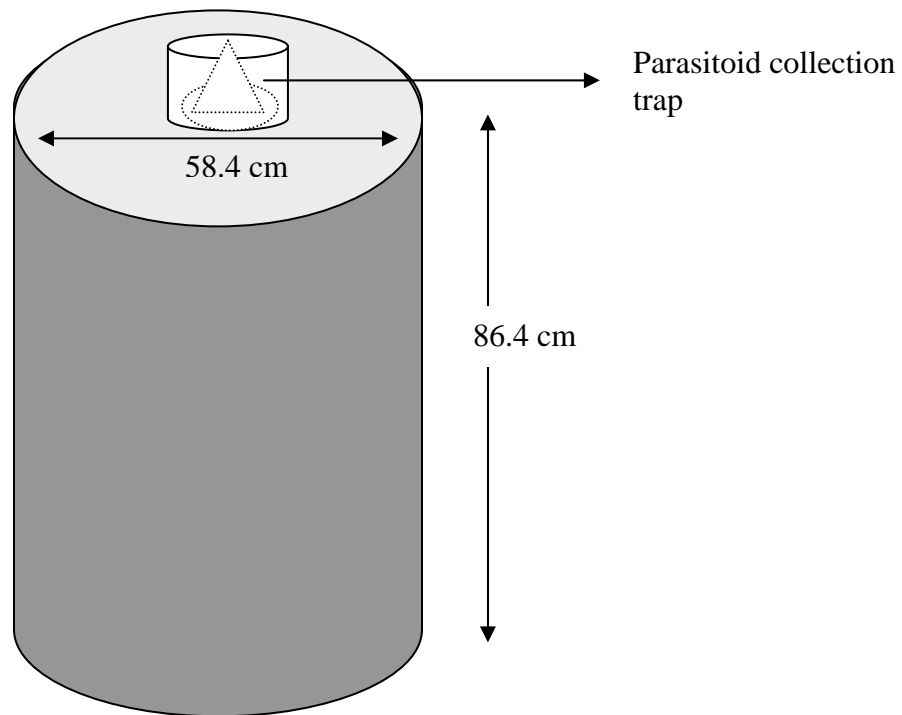


Figure 5. Diagram illustrating the emergence barrel used to collect emerging adult parasitoids (2004-05).



identical to those used for wheat. Canola flowered within 40 d, and retained flowers for 12 wk if pruned at pod set. Some of the sweet clover plants were vernalized for 20 d at 45 d after planting to induce early flowering. However, the vernalized and unvernallized plants flowered at the same time, at approximately 110 d after planting. Clover plants retained flowers for more than 14 wk, even after the plants were removed from the cages. Three sweet clover and two canola plants were transplanted to each of six field cages. Small holes of 14-cm diameter were dug between the wheat rows inside the rearing cages and the potted flowering plants were embedded in the soil at the level of the soil surface. This aided the retention of moisture in the pots for longer periods. The flowering plant species were irrigated three times a week. Honey or sugar in aqueous solution was also provided as a supplementary food source for the parasitoids in six cages receiving the flowering species. The solutions were hung in vials just above the crop canopy at five different locations inside the rearing cage—three in the center, and one at each end of the cage. These vials were filled and replaced three times each week. The specific treatments were.

- 1) Cages with windows and flowering plants, with the supplemental honey or sugar in aqueous solution.
- 2) Cages with windows, but without any supplemental food sources for the parasitoids.
- 3) Cages with supplemented food in the form of flowering plants and honey or sugar in aqueous solution.
- 4) Control cages having neither windows nor supplementary food.

The wheat plants inside the cages were grown to Zadoks 32, when they were infested with WSS on 1 July, 2004. The WSS used for infestation were laboratory reared and were 0-1 d old. The numbers were supplemented by field sweeping adult WSS of unknown age, near Churchill, Montana. The laboratory reared WSS were obtained from wheat residue from the previous season at Churchill, MT and the emerging adults were gently collected using a powered vacuum aspirator. WSS were then released sequentially on the same day into all cages. Each cage received 185 female and 105 male WSS. The flight activity and survival of the WSS were observed in the cages for the following week.

The WSS parasitoids, *B. cephi* and *B. lissogaster*, were released into the cages one month later, on 30 July, 2004. The parasitoids of unknown age were field-collected using sweep nets in on 'Ernest' spring wheat field near Ophiem, Montana on 29 July, 2004. The individuals were determined, sexed, and released late the next day into the mass rearing cages. The details are given in Table 5.

The parasitoid numbers were supplemented using newly-emerged adults obtained from wheat residue collected near Conrad, MT. These were reared out of a reinforced emergence barrel (Meers 2005), lined with a wire frame, and covered with black plastic sheeting on the sides to prevent the entry of light (Figure 5). The top of the barrel was covered with a white cardboard sheet with an 8 cm opening in the middle for the collection of parasitoids. The opening was covered with a translucent inverted conical funnel and a transparent collection cup. Light that entered through the opening attracted the emerging adult parasitoids which are trapped in the collection cup (Meers, 2005). The emergence barrels were incubated in a plastic greenhouse at the Horticulture Farm at

Montana State University, Bozeman. Parasitoid emergence was monitored daily. The newly collected parasitoids were sexed and released into the cages in equal amounts each day. The details for the newly emerged parasitoids that were released daily into the rearing cages from the barrels are given in Table 5. An average of 105 female parasitoids was released into each cage from the combined field collection and barrel emergence (Table 7).

All parasitoids inside the cages had died by the end of first week of September, approximately 40 d after release. At this time, the wheat plants were senescing in the field. The flowering plant species were removed and the cages were dismantled. This was followed by sample collection from the cages. Five samples comprising 53 cm² of planted area were taken from each cage. The sampling positions inside the rearing cages are shown in Figure 6. After sampling, the wheat plants inside the cages were combined by the farm manager at the Post Farm leaving wheat stubble at 50 cm height at the cage locations. The collected samples were dissected to determine the level of WSS infestation, the mortality of WSS, and the number of parasitized WSS. Contributions of each treatment to variation in infestation and parasitism were statistically analyzed using multivariate analysis of variance (MANOVA procedure of SAS [PROC GLM]). Infestation, parasitism and mortality were the multiple response variables. The number of wheat stems in each sample was a covariate in the analysis. Means and standard errors for each treatment were also plotted. The remaining wheat plants inside the individual cage

Table 5. Parasitoids collected from two different sources and released in different treatment cages at the Post Farm (2004).

Treatment cages	Parasitoids field collected from Ophiem on 30 July, 2004				Parasitoids collected from barrels during July 2004			
	<i>B. cephi</i>		<i>B. lissogaster</i>		<i>B. cephi</i>		<i>B. lissogaster</i>	
	Female	Male	Female	Male	Female	Male	Female	Male
Light and food source	90	13	15	6	0	0	90	13
Light and food source	93	9	15	8	0	0	93	9
Light and food source	94	4	9	3	2	2	94	4
Food source	89	3	14	1	0	1	89	3
Food source	91	2	15	0	0	2	91	2
Food source	89	5	11	3	0	0	89	5
Light	90	6	12	5	2	0	90	6
Light	94	3	16	1	0	0	94	3
Light	95	2	6	1	4	0	95	2
Control	88	3	13	2	0	0	88	3
Control	89	5	11	4	2	1	89	5
Control	93	5	8	4	2	0	93	5

Table 6. Parasitoids collected from two different sources to be released in different treatment cages for rearing parasitoids (2005).

Treatment cages	Parasitoids collected from Conrad on 20 July, 2005				Parasitoids collected from barrels during July 2005			
	<i>B. cephi</i>		<i>B. lissogaster</i>		<i>B. cephi</i>		<i>B. lissogaster</i>	
	Female	Male	Female	Male	Female	Male	Female	Male
Light and food source	91	1	0	20	7	3	0	0
Light and food source	91	1	0	17	7	4	0	0
Light and food source	93	1	0	15	7	7	0	0
Food source	92	2	0	16	5	0	0	0
Food source	93	0	1	9	4	5	0	0
Food source	96	0	0	12	6	0	0	0
Light	93	0	1	4	6	0	0	0
Light	93	0	0	18	6	2	0	0
Light	92	2	0	7	6	0	0	0
Control	98	1	1	12	4	0	0	0
Control	96	0	1	13	6	0	0	0
Control	90	0	0	27	6	0	0	0

Table 7. Total parasitoids released in different field cage treatments (2004-05).

Treatment cages	Total parasitoids in 2004				Total parasitoids in 2005			
	<i>B. cephi</i>		<i>B. lissogaster</i>		<i>B. cephi</i>		<i>B. lissogaster</i>	
	Female	Male	Female	Male	Female	Male	Female	Male
Light and food source	105	19	1	0	98	4	0	20
Light and food source	108	17	0	0	98	5	0	17
Light and food source	103	7	2	4	100	8	0	15
Food source	103	4	2	2	97	2	0	16
Food source	106	2	2	4	97	5	1	9
Food source	100	8	0	0	102	0	0	12
Light	102	11	4	0	99	0	1	4
Light	110	4	0	0	99	2	0	18
Light	101	3	6	0	98	2	0	7
Control	101	5	1	0	102	1	1	12
Control	100	9	4	2	102	0	1	13
Control	101	9	2	0	96	0	0	27

areas after sampling and harvest were collected and placed inside emergence barrels to enumerate parasitoid emergence the following spring.

Parasitoid Mass Rearing (2005): The field rearing experiment was repeated in 2005 with several modifications suggested by the results from the previous year. The more susceptible wheat variety 'Reeder' was planted to replace 'McNeal'. The wheat rows were planted from north to south direction and the 12 cages were set up with their long axes oriented north to south to capture maximal sunlight. This planting and cage orientation also reduced the physical damage to the wheat that occurred while working inside the cages. There were six rows of wheat planted in each cage. The four treatments used in 2004 were repeated in 2005. Once again each treatment had three replications randomly assigned among the 12 cages. The layout of the cages in the field is shown in Figure 3.

Three flowering plant species, canola, yellow sweet clover and birdsfoot trefoil (*Lotus corniculatus* L.) were used as a nectar source for the parasitoids. Once again, canola and yellow sweet clover were raised in greenhouse and were transplanted into the field after flowering. The sweet clover flowered inconsistently, while the canola flowered rapidly, after approximately 6 wk. The requirement for supplemental flowering plants was met by transplanting birdsfoot trefoil plants that were growing wildly near the MSU Marsh Laboratory in Bozeman, MT. These were immediately transported to the greenhouse and planted in 15 liter pots with the soil mix used for growing the experimental wheat plants. These were watered daily until vigorous growth resumed. After monitoring for a week, the vigorous plants were taken to the rearing cages along

with the flowering pots of canola and sweet clover. The transplanting and supplemental provisioning were added to the 2004 experimental protocol, and all other aspects of the experimental treatments remained the same.

The method of releasing WSS into the mass rearing cages at Zadoks growth stage 32 was modified. Stubs from 'McNeal' spring wheat grown near Churchill in 2004 are used as the source of WSS for the cage experiments. Groups of intact, healthy stubs from the same population were monitored in the laboratory for emergence rates and sex ratio. This provided an estimate of the required number of stubs for each rearing cage. Based on these results, 500 stubs were placed inside each cage. They were kept in a 51 x 25 x 5 cm tray which had screened sides and coarse mesh bottom for water drainage and air circulation. This tray was placed between the wheat rows. The tray was shaded by the wheat canopy and raised from the ground by the soil mounds that supported the edges to facilitate water drainage. The WSS adults started emerging from the stubs when the wheat reached Zadoks growth stage 38 on 4 July, 2005. The flight activity of WSS was observed each day for 2 wk. After the WSS emergence ended, the stubs used in each cage were sorted and dissected to determine the number of WSS that emerged. These emergence data and the laboratory data on the sex ratio were used to estimate the number of female WSS in each cage.

The WSS parasitoids *B. cephi* and *B. lissogaster*, were released into the cages on 21 July, 2005. The majority of parasitoids were field collected on 20 July, 2005 using sweep nets in 'Reeder' spring wheat grown near Conrad, MT. The individuals were determined, sexed, and released the next day into the rearing cages in the numbers shown in Table 6.

As in 2005, parasitoid numbers were supplemented by newly-emerged individuals that were obtained from wheat samples collected from the rearing experiment in 2004. These were collected and distributed as per the 2004 experiments in the numbers shown in Table 6. An average of 99 female parasitoids was released into each cage from the field and barrel emergence, which was fewer than the number released during the previous experiment (Table 7).

All parasitoids were dead inside the cages by the end of August, which was 45 d after their release. At this time, the wheat plants had started to senesce in the field cages. Once again, flowering plants were removed and the cages were dismantled, followed by sample collection from the cages. Nine samples of 48 cm² crop area were taken from each cage. The sampling locations inside the rearing cages are shown in Figure 7.

After sampling, the wheat plants inside the cages were combined by the farm manager at the Post Farm leaving wheat stubble at 50 cm height at the cage locations. The collected samples were dissected to determine the level of WSS infestation, the mortality of WSS, and the number of parasitized WSS. Contributions of each treatment to variation in infestation and parasitism were statistically analyzed using multivariate analysis of variance (MANOVA procedure of SAS [PROC GLM]). Infestation, parasitism and mortality were the multiple response variables. The number of wheat stems in each sample was a covariate in the analysis. Means and standard errors for each treatment were also plotted. The remaining wheat plants inside the individual cage areas after sampling and harvest were collected and placed inside emergence barrels to enumerate parasitoid emergence the following spring. The parasitoids emerging from the

wheat residue were more abundant in 2005 and were analyzed for treatment differences in the number of parasitoids and sex ratio.

Figure 6. Sample collection areas inside the mass rearing cages at the Post Farm (2004).

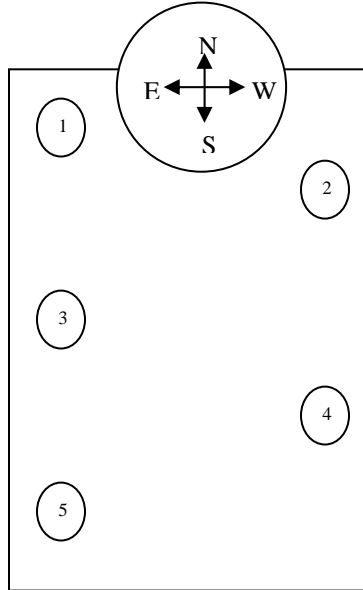
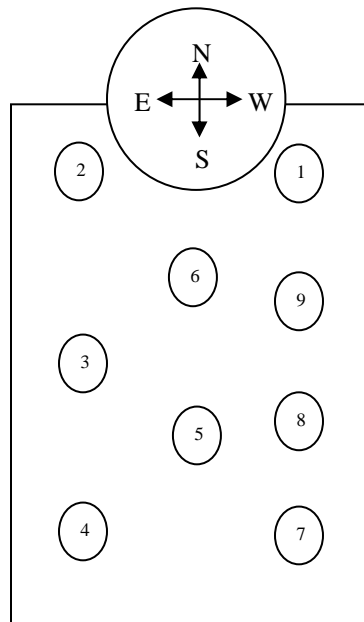


Figure 7. Sample collection areas inside the mass rearing cages (2005).



WSS Infestation Variation as Influenced
by the Method of Introduction (Objective 2)

When the parasitoid mass rearing experiments were conducted for the second season at the Post Farm, the method of introducing the WSS adults into the rearing cages was modified. This was done to determine if different methods of introduction would influence the infestation levels by WSS in wheat. The host levels would limit the amount of parasitism in the mass rearing cages and could be more significant than the availability of other factors like light and food sources for the larval parasitoids.

For the first season (2004), the wheat plants inside the cages were grown to Zadoks 32, when they were infested with WSS on July 1, 2004. The WSS used for infestation were laboratory reared and were 0-1 d old. The numbers were supplemented by field sweeping near Churchill, Montana. The laboratory reared WSS were obtained from wheat residue of the previous season from Churchill, MT and the emerging adults were gently collected using a tube connected to a vacuum pump. The sawflies were then released sequentially on the same day into all cages. Each cage received 185 female and 105 male WSS. The flight activity and survival of the WSS was observed in the cages for the following week.

For the second season (2005), the method of releasing WSS into the mass rearing cages at Zadoks growth stage 32 was modified. Stubs from 'McNeal' spring wheat grown near Churchill in 2004 was used as the source of WSS for the cage experiments. Groups of intact, healthy stubs from the same population were monitored in the laboratory for emergence rates and sex ratio data. This provided an estimate of the required number of stubs for each rearing cage. Based on these results, 500 stubs were

placed inside each cage. They were kept in a 51 x 25 x 5 cm tray which had screened sides and coarse mesh bottom for water drainage and air circulation. This tray was placed between the wheat rows. The tray was shaded by the wheat canopy and raised from the ground by the soil mounds that supported the edge to facilitate water drainage. The WSS adults started emerging from the stubs when the wheat reached Zadoks growth stage 38 on July 4, 2005. The flight activity of WSS was observed each day for 2 wk. After the WSS emergence ended, the stubs used in each cage were sorted and dissected to determine the number of WSS that emerged. These emergence data and the laboratory data on the sex ratio were used to estimate the number of female WSS in each cage.

Size and numbers of wheat stem samples collected for the two seasons were different. The information on the number of infested stems from the total samples collected from a mass rearing cage was extrapolated to 1000 wheat stems for standardization. This was used to estimate the number of wheat stems a WSS female would infest in each treatment cage for the two seasons. There were 185 females in each cage for the first season, but the second season had variable emergence and with a possible variability in sex ratio for each cage. A bar diagram was created to illustrate differences in the number of stems infested by a WSS female for both seasons.

High Temperature Mortality Studies (Objective 3)

Temperature Variation in the Wheat Canopy

The experiment was conducted in the greenhouse to determine the potential mortality in parasitized WSS larvae at high temperatures. To accomplish this, temperature measurements were taken in the wheat canopy, both within and outside the

stem at different heights along the growing plant. This will assess the possibility that the WSS larvae inside the stems are protected from the high temperatures that might occur in the field.

The study was conducted at the MSU Plant Growth Center under the greenhouse experimental conditions described previously. Four pots of soil were each planted with four seeds and grown in the greenhouse until they were four weeks past the Zadoks (Nelson *et al* 1988) 32 stage (in Zadoks 45 stage) because the wheat plants are usually infested at the Zadoks 32 stage. Six supplemental bulbs (120W General Electric Plant Gro N Show) were positioned over the four wheat pots. The heat sources were at 25 cm above the top of the wheat canopy so that the plants were not heat scorched. The wheat plants were continuously heated for 5 hr and the temperature readings were taken at the end of the exposure period. A Model 600-1020 type T thermocouple thermometer (Barnant, Union City, CA) with an extended measuring probe was used to determine the temperature readings inside the stem, and a Model 42529 Infrared non-contact thermometer (Extech Instruments, Waltham, MA) was used to acquire temperature readings outside the stem. The inside and outside stem temperature measurements were taken at heights of 42, 32, 22, 10, and 0 cm from of the soil surface along the primary tiller of 8 wheat plants, at a high canopy temperature. The mean and standard error of the temperature readings at the different distances were determined and a graph was plotted for the paired temperature readings inside and outside the wheat stem at the different heights.

Potential WSS Larval Mortality in Stems
at High Temperatures in the Greenhouse

Eight pots of 'McNeal' spring wheat were raised inside the greenhouse as above. At Zadoks 32, eight of the wheat plants (one per pot) were randomly selected from the group of 32 plants for infesting with WSS. The primary tillers of each of the eight plants were caged using plexiglass tubes to establish infestation. The tube had three openings of 5 cm diameter covered with mesh allowing aeration, and another opening of 1 cm diameter plugged with cork, for introduction of adult WSS. The plexiglass tube was placed over the primary tiller, with all the leaves inside. The leaves were held straight and care was taken not to damage them. The base of the tube was embedded in the soil encircling the plant base. A bamboo stake was also firmly embedded in soil, and tied to the adjacent tube to hold the tube in position.

Newly emerged WSS adults were used for infestation. These were obtained from the 'Conan' spring wheat stubs collected near Conrad, Montana in the spring of 2004. The WSS adults were extracted from an emergence container using a suction tube and held in glass jars. Two female and one male WSS adults were released into each of the eight tubes for infesting the primary tillers. The WSS were maintained inside the tube until they died, approximately one week later. The tubes were then removed and the plants were grown in the greenhouse for 4 wk (until they reached the Zadoks 45 stage) after the initial release of the insects. This method assured that 3 to 4 wk-old WSS larvae would be exposed to the high experimental temperatures that could match the conditions in the wheat field. At this time, the wheat plants were arranged in two rows of four pots

and the six heat bulbs used in the previous experiment were again positioned at a height of 25 cm above the wheat plants and heated continuously for 5 hr on each of 2 d.

The above two experiments using the infested and uninfested wheat plants were conducted separately to eliminate the possibility that probing to measure the internal stem temperatures could result in larval WSS mortality. External temperatures were verified and found to be similar to the previous experiment. After exposing the wheat plants to 5 hr of high temperature for two consecutive days as described above, the mortality and position of the larva within the eight stems was determined immediately after the exposure period. Determinations were made by dissecting the stems lengthwise using an X-ACTO[®] knife (Hunt Corp., Statesville, North Carolina). This experiment also allowed the determination of potential behavioral avoidance of high temperatures by unparasitized larval WSS.

Temperature-induced Mortality of Overwintered WSS in Stubs in the Greenhouse

Rationale for Using WSS Larvae in Diapause: Wheat ‘stubs’ are the lower half of the WSS-cut wheat stems and contain the inactive larvae in a hibernaculum. The results from the previous study show that active larvae move away from higher temperatures, so constraining these in place as a surrogate for parasitized larvae may be a poor substitute for an immobilized, paralyzed larva. In addition, it may be limiting to obtain field-collected, parasitized WSS larvae with similar stages of actively-feeding parasitoids in adequate numbers for experimentation by dissecting wheat stems. Therefore, because WSS larvae undergo an obligatory diapause from late summer until next May, these may

be a useful model for assessing temperature-induced mortality. In addition, when diapause is broken by the development of pre-pupal stage ambient field temperatures are rising. However, even before the development of the pre-pupa in early May, the hibernating larva inside the wheat cut stem could be exposed to high temperatures as the postharvest residue warms. These immatures might be exposed to higher temperatures than those found in growing wheat plants due to the absence of a buffering canopy. This could result in a higher mortality of the larvae in these overwintered stubs when compared to larvae in growing plants. The restriction of larval movement inside stubs and the proximity to the warm soil surface during spring and early summer development also might be more conducive to mortality. This experiment was conducted in the greenhouse to determine the mortality of the diapausing larva in the wheat stubs as a potential contribution to overall population dynamics and as a model for potential mortality of paralyzed larvae in the growing wheat stem, when exposed to high temperatures.

The stubs for the experiment were collected from a heavy WSS infestation in 'Reeder' spring wheat near Conrad, MT. The experiment was conducted in the greenhouse that was described earlier. Three trays of size 53.0 x 28.0 x 7.6 cm were filled with the Plant Growth Center soil mix. A control tray was kept at the greenhouse daytime temperature of 22 °C, while the stubs in the other trays were exposed to the elevated temperature. The stubs were buried in the soil so that the top half is above the ground level. Six heat bulbs (GE, 120W, Plant Gro N Show, Charles Walters, Sheridan, MT) were positioned 60 cm above the temperature treatment trays. Each tray contained 30 stubs and these were exposed to moderately high temperatures averaging 29 °C for 6 hr. At the end of exposure period, three readings were taken outside 10 randomly selected

stubs (a) one set of 10 stubs at one cm above the soil surface (b) one set of 10 stubs on the soil surface (c) and one set of 10 stubs at one cm below the soil surface, using a type T copper-constantan thermocouple thermometer (Barnant, Union City, CA). After the temperature measurements were taken, the stubs were kept at room temperature for a period of 24 hr, and were dissected using an X-ACTO[®] knife for recording mortality. The larvae were probed with a blunt needle to determine mortality.

Pupae: The diapausing larva of WSS pupates in the late May as environmental conditions warm. The pupal stage becomes an adult in another 2 wk. This stage lacks mobility and might be vulnerable to more frequent elevated temperatures in comparison to the diapausing larva. The brown-eyed pupal stage (Ainslie 1920) was selected for the experiment.

The stubs containing WSS for the experiment were collected from Churchill, Montana in the fall of 2005. Three trays of size 53.0 x 28.0 x 7.6 cm were filled with Plant Growth Center soil mix. The sterile soil was placed inside the tray at a depth of 5 cm and the stubs were embedded at a depth of 2 cm in the soil, so that the top half was above the ground level. The cut-stems were arranged in each tray so that at least 1 cm was projecting above the soil surface. The stubs were arranged in five rows of 10 in each tray. Six heat bulbs, as previously described, were used as heat sources and were positioned 60 cm above the soil surface of two treatment trays while a control tray was kept at room temperature ranging from 18 to 22 °C. The temperature readings were again taken using a type T copper-constantan thermocouple thermometer (Barnant, Union City, CA). Slightly different methods were followed to understand the temperature distribution

in the stubs and the exposure of the WSS pupa compared to the earlier experiment using diapausing larvae.

Temperature readings were recorded at three different positions: (a) the air temperature outside the stub just above the frass plug (b) air temperature inside the top half of the stub (c) air temperature inside the bottom half of the stub. The extended probe of the thermocouple thermometer was used to note the readings inside the cut stem through the frass plug without damaging the stub. The bottom half of the wheat cut-stem will be in contact with the heated layer of soil which might result in higher temperature levels inside the stub at this position. Temperature readings at these three positions were recorded from 25 stubs in each tray. Inserting the probe of the thermocouple thermometer into the stub might have injured the WSS larvae and caused mortality, so the stubs which were used for temperature measurements were excluded from the mortality data. The temperature readings were taken while the stubs embedded in the soil were being exposed to high temperatures. The remaining stubs from the trays were kept at room temperature for 24 hr. They were then taken to the lab and split using an X-ACTO[®] knife and mortality of the WSS pupa was recorded. The pupae were probed with a blunt needle and observed under bright light to observe activity or mortality. The percentage mortality was compared to that in the control where the stubs were not exposed to high temperature. This study was replicated.

The experiment was also conducted by placing the heat sources at a height of 90 cm so that the effect of moderately high temperature levels could also be measured. This study was otherwise conducted identically to the study described above with the heat sources at 60 cm.

Temperature Variation in the Field at the WSS Pupal Stage

Experiments were conducted in the field to characterize the temperature regime that the WSS pupae would be exposed to in stubs. Four types of temperature measurements were performed: (a) the ambient air temperature (b) the soil temperature at a depth of 1 cm (c) the soil temperature at a depth of 3 cm (d) the temperature inside the stub at a depth of 1 cm when its frass-plugged surface is just above the soil surface. Air temperature was measured using an infrared non-contact thermometer while the soil and within stub temperatures were measured using a type T copper-constantan thermocouple thermometer. The temperature measurements were initiated at 1400 hours on 18 May, 2006 at the Post Farm. On this date the ambient temperature was atypically high for this time of year and at this time, the field population of WSS is mostly in the brown-eyed pupal stage. Temperature measurements were taken at 50 different positions beneath the soil surface at the two different depths, and 50 sequential air temperature readings were recorded, each separated by a time interval of one minute. Temperatures were also recorded at 50 different locations just below the soil surface while keeping the extended probe of the thermocouple thermometer inside the stubs. A bar graph was plotted with means and standard errors for each of the four temperature categories. The results emphasized the importance of mortality studies on WSS at high temperature levels.

A second experiment was conducted at the Post Farm when the air temperature was more moderate. Temperatures were recorded as described above. The temperature measurements were initiated at 1400 hr on 24 May, 2006, when some of the field

population of WSS would still be in the brown eyed pupal stage. Once again, the data was plotted as means and standard errors for each of the four temperature categories.

Laboratory: LTe₅₀ of WSS Larvae and Pupae

The lethal temperatures (LTe) required to cause 50% mortality in the experimental populations were collected from larval and pupal WSS from the 'Reeder' and 'McNeal' spring wheat fields infested in 2004 and 2005, respectively. Both stages were assessed after exposure to an obligatory diapause of at least 90 d.

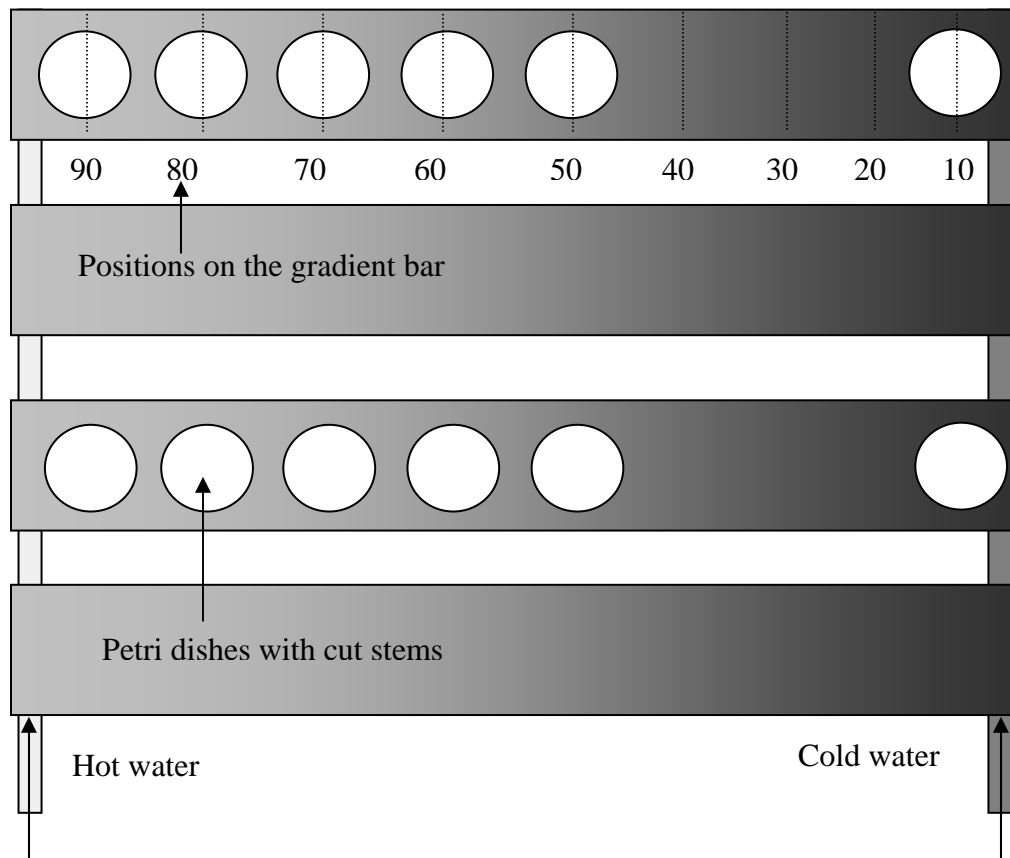
Diapausing Larvae: Stubs from the spring wheat variety 'Reeder', collected from a field near Conrad, Montana, in the spring of 2005, were used for the experiment. Based on the results from the greenhouse experiments on high temperature exposure of diapausing WSS larva in stubs, more data were collected in a set of experiments to better describe temperature-induced mortality across a range of elevated temperatures. A laboratory thermal gradient was used to obtain a range of elevated temperatures. The gradient consists of four parallel 100 x 15 x 1 cm thick aluminum bars. The ends of each bar were placed on hollow aluminum rods which run perpendicular to the four gradient bars. The system also includes two tanks, one of which circulates hot water at one end of the bars in contact with the hollow rods while a 50:50 propylene glycol and water mixture from the other tank was circulated through the hollow rod in contact with the other end of the bars (Figure 8). This helps to maintain a linear temperature gradient along the four bars, varying not more than 1.5 °C at parallel positions across the four bars. There is also a lid of plexi glass protecting the horizontal bars from air currents to limit variation in temperature.

Before starting the experiment, the equipment was operated for 4 hr to stabilize the temperature. Field-collected stubs were cleaned and the roots were removed. These were then placed inside Petri dishes. A filter paper was placed at the bottom of each Petri dish. The relative humidity inside the Petri dish was maintained between 81 – 85% and was recorded using a HOBO[®] H8 RH/Temperature data logger (Onset Computer Corporation, Pocasset, MA). Four stubs were placed in each of the Petri dishes on moistened filter paper. The sides of the Petri dish were sealed using parafilm, so that moisture levels would be maintained within the dish. Six Petri dishes were placed on each of the four gradient bars at identical positions so that the temperatures were effectively constant at these positions across the four bars. The experiment was replicated three times. The six different temperatures that the stubs were exposed to were 54, 48, 44, 41, 39, and 37 °C. These were determined using an infrared non-contact thermometer (Extech Instruments, Waltham, MA). Temperature was noted at the end of each exposure time interval. At each temperature point and a specific time interval there were 48 stubs considering the replications in time and space. The time intervals selected were 2, 3, 4, and 5 hr for which separate experiments were conducted to avoid covariance associated with correlations in mortality over time as described by Throne et al. (1995a).

After the specific time interval of an experiment was concluded the Petri dishes were removed from the gradient and kept at room temperature for 24 hours. The stubs were then dissected and the mortality of the diapausing WSS larvae inside the stubs was assessed. The mortality was determined by probing the larvae with a blunt needle under bright light to observe the presence or absence of activity. Lethal temperatures for 50% of the population (LT₅₀) with fiducial limits and Pearson chi-square goodness-of-fit values

were determined for each of the four time intervals. The data were analyzed (Throne et al. 1995a) by using the Probit procedure in SAS[®] (SAS 1990), with transformed mortality data assessed against a logistic distribution. The fitted line was then backtransformed in Mathematica[®] using the programs described in Throne et al. (1995b). Graphs were plotted using the backtransformed predicted curves and observed mortality data.

Figure 8. Thermal gradient equipment used to determine WSS temperature-induced mortality.



Pupal Stage: A similar set of experiments were conducted using the brown-eyed pupal stage of WSS. The gradient used was similar to that used for the previous experiment, but also had embedded thermocouples and a digital display that gave

temperature readings at the 10 equally-spaced points along each of the four gradient bars (Figure 8). The wheat stubs used for this experiment were collected from Churchill, Montana in the fall of 2005 and stored at 4 °C for 110 d before warming at room temperature. They were exposed to room temperature for 18 d to break diapause and initiate pupation. Each week, samples were taken to determine the age of the larvae or pupae to ensure developmental uniformity for the experiment.

Before starting the experiment, temperature readings on the gradient bar surface and the variation in temperature inside the stub at different positions along the gradient bar were measured (Figure 9 and 10). The stubs were placed inside a Petri dish on a lightly moistened filter paper, which maintained a relative humidity ranging from 80 to 85% inside the Petri dish, as described above. The probe of the type T thermocouple thermometer was placed inside the stub through the frass plug located in the center of the Petri dish. The dish was then sealed using parafilm (Parafilm “M”, American Can Company, Greenwich, CT) to prevent the escape of moisture and reduce the loss of heat in air currents. Readings inside the stub were taken using the thermocouple thermometer after the temperature became stabilized both on the gradient bar and inside the Petri dish.

The temperature gradient was set to equilibrate the temperature at the six selected positions to 49, 46, 44, 42, 40, and 36 °C. Based on these results, a seventh temperature of 55 °C was selected where high mortality of WSS pupae was confirmed. The gradient bars were set for these temperature and readings were taken for four different time intervals with three replications over time, as described for the overwintered larvae. After the stubs were exposed to the seven temperatures and four separate time interval experiments, they were removed from each Petri dish and placed in separate vials

plugged with moistened cotton covered in close-meshed cloth. The frass plugged portion of the stubs was held facing the cotton plug to facilitate WSS emergence and were moistened twice weekly. The vials were arranged horizontally on a 63.5 x 43 x 5 cm tray and exposed to daylight at room temperature until adult emergence ceased.

The development time in days, presence of abnormal adults and mortality of the WSS pupae were recorded. Lethal temperatures for 50% of the population (LT_{50}) with fiducial limits and Pearson chi-square goodness-of-fit values were determined for each of the four time intervals. The data were analyzed (Throne et al. 1995a) using Probit procedure in SAS[®] (SAS 1990), with transformed mortality assessed against a normal distribution. The fitted lines were backtransformed (Throne et al. 1995b) and plotted as described above.

Figure 9. Temperatures at different positions on the thermal gradient bars illustrated in Figure 8.

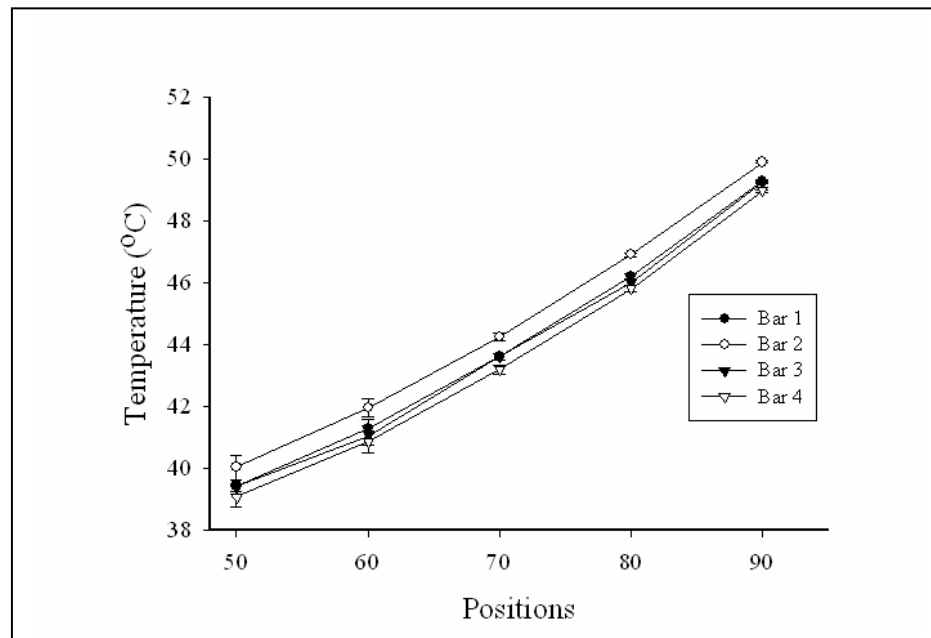
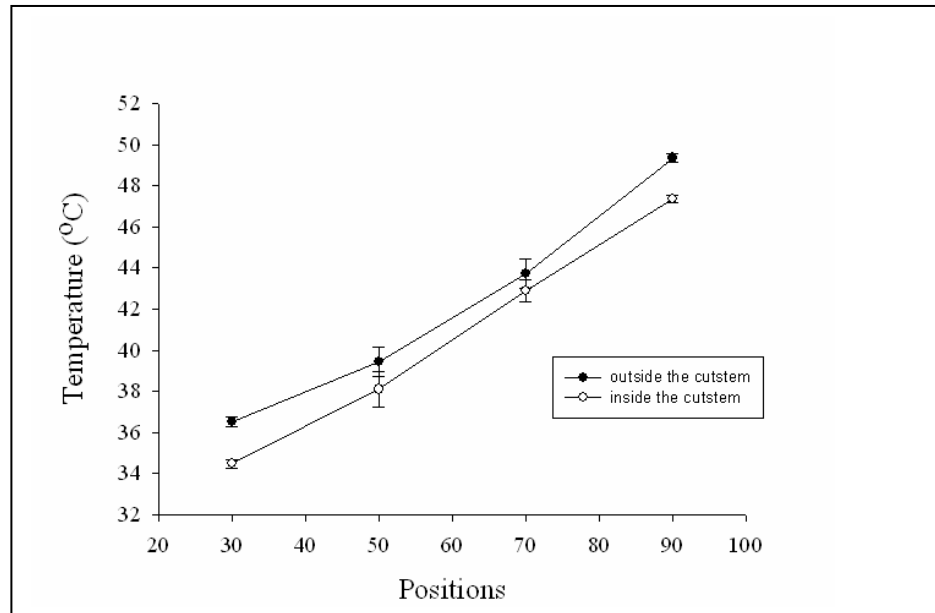


Figure 10. Temperature inside and outside wheat stubs placed on the thermal gradient bars.



Laboratory: LT₅₀ of Sawfly Larvae and Pupa

Lethal times to cause 50% mortality in the experimental population (LT₅₀) were also calculated, while keeping the temperature and stage of the wheat stem sawfly constant and collecting mortality data over varying one hour time increments. There were three experimental replications for each time interval at the selected constant temperature for both the WSS immature stages. Lethal time for the WSS diapausing larval mortality at 48 °C was calculated using mortality data from four separate time intervals-2, 3, 4, and 5 hr. The same criteria were used to assess mortality over time at 49 °C for the brown-eyed stage of WSS pupae. LT₅₀ values with fiducial limits and Pearson chi-square goodness values were determined using the Probit procedure (Throne et al. 1995a) in SAS[®] (SAS

1990), with mortality transformed for assessment against a logistic distribution. The fitted line was also backtransformed (Throne et al. 1995b) and plotted as previously described.

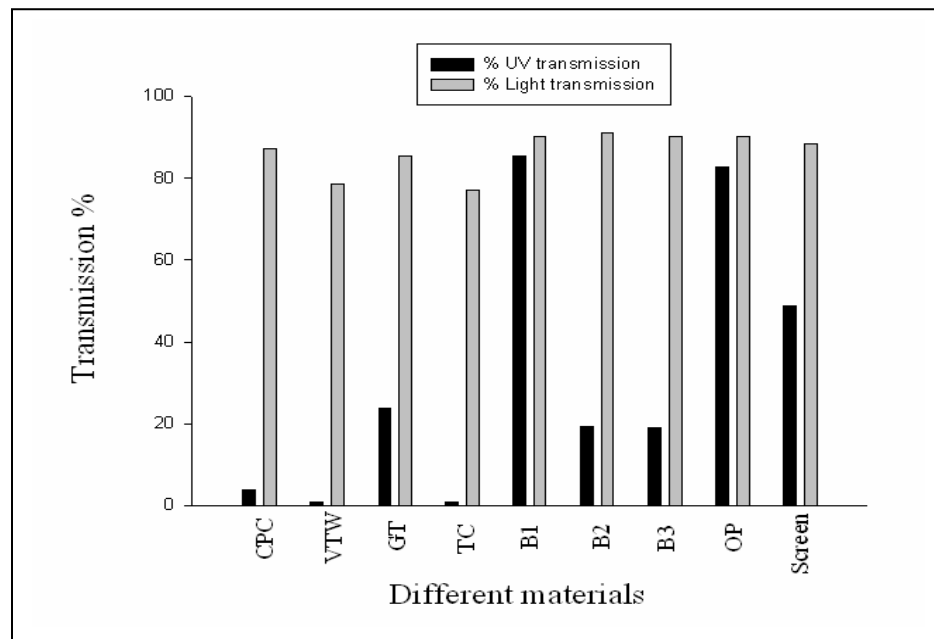
CHAPTER THREE

RESULTS

Mass Rearing of *B. cephi* and *B. lissogaster* (Objective 1)Infestation and Parasitism
in Greenhouse Cages

Selection of UV Transmitting Material: The ultraviolet and visible light measurements for the nine materials are plotted (Figure 11). The transmission percentages were calculated when the light and UV sensors were at a distance of 10 cm from the material surfaces under consistent conditions of illumination.

Figure 11. Per cent of UV and visible light transmission for Corrugated PC sheet (CPC), GT 6 mil IR/AC (GT), Lexan Thermoclear® TC2 112 (TC), Barlo XT-1 (B1), Barlo XT-2 (B2), Barlo XT-3 (B3), Acrylite® OP4 acrylic sheet (OP), Cage covering with 530µm mesh openings (Screen). The details for the materials are given in the text.



The Barlo XT-1 product, a form of extruded polymethyl methacrylate, showed the highest percentage of ultraviolet and visible light transmission, but was commercially unavailable in North America. The second best material was the Acrylite[®] OP4 acrylic sheets (Cyro Industries, Chicago, IL) which transmitted 83% of the ultraviolet and 90% of visible light at the tested distance of 10 cm below the material and was selected for use as windows in the mass rearing cages. The existing cage mesh, the amber lumite screen 7250Q with 530 μ pore openings (BioQuip Products, Rancho Dominguez, CA) cage material transmitted 88% of the visible light and 49% of the UV light. Thus, cages assembled with the Acrylite[®] OP4 acrylic windows would be expected to have approximately 90% greater levels of ultraviolet light and similar amounts of visible light.

Rearing in Greenhouse Cages: The infestation and parasitism for the six treatments are given in Table 8. The percent infestation was greater in cages with greater amounts of visible light, and no infestation was found in cages without the enhanced visible light. The impact of the other factors on sawfly infestation could not be evaluated using the collected data. Even though a low level of parasitism was seen in the cages with supplementary visible light, the preliminary data were not robust enough to draw conclusions. A second experiment was planned for 2005 based on the fact that light appeared to be the most important factor affecting sawfly infestation and resulting parasitism. The infestation and parasitism for the three treatments in the second experiment are given in Table 9.

Table 8. Infestation and parasitism numbers in greenhouse rearing cages (2004).

Treatments	No. of stems splitted	Infested	Parasitized
Light	17	10	2
Light, Food source	16	1	1
Light, UV	14	1	1
Light, UV, Food source	13	6	0
Food source	12	0	0
Control	13	0	0

Table 9. Infestation and parasitism numbers in greenhouse rearing cages (2005).

Treatments	Replication	Total stems	Infested	Parasitized
Light, UV	1	15	0	0
	2	16	1	0
	3	15	1	0
Light	1	16	0	0
	2	15	1	0
	3	16	0	0
Control	1	17	1	0
	2	17	0	0
	3	18	0	0

Each treatment had three replications. Due to insufficient infestation, the treatments could not be statistically analyzed. The reasons for the failure to achieve infestation are unknown, but a percentage of greenhouse experiments using the WSS

consistently fail. Due to the prolonged experimental period, it was not possible to undertake additional greenhouse experiments after stem dissection at plant maturity revealed the lack of successful infestation.

Mass Rearing of Parasitoids in Field Cages

Temperature Variation (2004-05): Significant contributions to the variation in temperature were due to differences inside or outside the cages, the type of cage (with or without windows), and the time when the observations were taken for the year 2004 (overall model: $F = 37.8$; $df = 17, 558$; $P < 0.01$). For 2005, there were significant contributions to the variation in temperature due to differences inside or outside the cages, the position inside the cages, and the time when the observations were taken (overall model: $F = 142.1$; $df = 10, 949$; $P < 0.01$). The models were fit with only those individual parameters that were significant at $P < 0.05$.

When temperatures inside the cages were analyzed separately, temperature variation was found to significantly differ with the type of cage (with or without windows), position inside the cages, and the time when the observations were taken (overall model: $F = 31.3$; $df = 16, 463$; $P < 0.01$) for the year 2004. For 2005, significant differences in temperature variation were seen only for the cage type (with or without windows) and time when the observations were taken (overall model: $F = 10.2$; $df = 16, 271$; $P < 0.01$). The models were fit with only those individual parameters that were significant at $P < 0.05$. The means and associated standard errors for the temperature measurements for 2004 and 2005 are reported in Table 10.

Visible Light Variation (2004-05): Significant contributions to the variation in visible light intensity were due to differences between measurements inside or outside the cage and the type of cage (with or without windows) (overall model: $F = 45.1$; $df = 17$, 941 ; $P < 0.01$). For 2005, significant contributions to the variation in visible light were due to differences in intensity inside or outside the cages, the type of cage (with or without windows), and the time when the observations were taken (overall model: $F = 84.7$; $df = 17$, 557 ; $P < 0.01$). The models were fit with only those individual parameters that were significant at $P < 0.05$.

Table 10. Means and associated standard errors of temperature ($^{\circ}\text{C}$), inside and outside the mass rearing cages at the MSU Post Farm. Variation for the two cage types is also shown (2004-05).

Inside/Outside cage	Cages without windows	Cages with windows
August 2004		
Inside temperature	25.3 (0.4)	26.1 (0.3)
Outside temperature	28.7 (0.4)	28.7 (0.4)
August 2005		
Inside temperature	23.2 (0.4)	23.4 (0.4)
Outside temperature	26.8 (0.4)	27.2 (0.4)

When the light intensities inside of the cages were analyzed separately, significant contributions to the variations were due to the type of cage (with or and without windows), and the position inside the cages (overall model: $F = 10.5$; $df = 16$, 462 ; $P < 0.01$) for the year 2004. For 2005, significant contributions to the variation in visible light

were due to the type of cage (with or without windows), position inside the cages, and the time when the observations were taken (overall model: $F = 11.2$; $df = 16, 271$; $P < 0.01$). The models were fit with only those individual parameters that were significant at $P < 0.05$. The means and associated standard errors for the light measurements recorded in 2004 and 2005 are reported in Table 11.

Table 11. Means and associated standard errors of light measurements (W m^{-2}), inside and outside the mass rearing cages at the MSU Post Farm. Variation for the two cage types is also shown (2004-05).

Inside/Outside cage	Cages without windows	Cages with windows
August 2004		
Inside light	396.3 (7.7)	565.8 (14.2)
Outside light	791.0 (13.7)	827.8 (13.0)
August 2005		
Inside light	462.7 (10.2)	585.5 (13.7)
Outside light	882.6 (9.2)	884.8 (7.9)

Ultraviolet Light Variation (2004-05): For the ultraviolet light measurements in 2004, there were significant contributions to the variation due to differences between inside or outside cage measurements, the type of cage (with or without windows), and the time when the observations were taken (overall model: $F = 67.8$; $df = 17, 750$; $P < 0.01$). For 2005, significant contributions to variation were due to differences between inside or outside cage measurements, type of cage (with or without windows), and the time when

the observations were taken (overall model: $F = 112.4$; $df = 17, 558$; $P < 0.01$). The models were fit with only those individual parameters that were significant at $P < 0.05$.

When the ultraviolet light intensities inside of the cages were analyzed separately, a significant contribution to the variation was only due to the type of cage (with or without windows - $F = 9.5$; $df = 16, 367$; $P < 0.01$) for the year 2004. For 2005, significant contributions to the variation in ultraviolet light were due to the type of cage (with or without windows), position inside the cages, and the time when the observations were taken (overall model: $F = 18.6$; $df = 16, 271$; $P < 0.01$). The models were fit with only those individual parameters that were significant at $P < 0.05$. The model for 2005 was fit with only those individual parameters that were significant at $P < 0.05$. The means and standard errors of the ultraviolet measurements are given in Table 12.

Table 12. Means and associated standard errors of ultraviolet measurements (mW cm^{-2}), inside and outside the mass rearing cages at the MSU Post Farm. Variation for the two cage types is also shown (2004-05).

Inside/Outside cage	Cages without windows	Cages with windows
August 2004		
Inside UV	0.49 (0.01)	0.77 (0.03)
Outside UV	1.38 (0.03)	1.39 (0.03)
August 2005		
Inside UV	0.46 (0.01)	0.72 (0.02)
Outside UV	1.35 (0.02)	1.37 (0.02)

Parasitoid Mass Rearing (2004): Infrequent flight activity was observed inside the cages after adult WSS were introduced, obtained through emergence in laboratory or by

field sweeping, were released to infest the wheat. The samples collected from the cages were processed to determine the amount of WSS infestation and parasitism. Very few wheat plants were infested by WSS inside the rearing cages, which matched the visual observations on flight activity. The few infested wheat plants were mostly parasitized. Means and associated standard errors for the amount of and percent of infestation and parasitism per sample are reported for the different treatments (Tables 13 and 14).

Table 13. Sample means and associated standard errors of infestation and parasitism (SE) for different rearing cage treatments (2004).

Treatment	Number of stems split	Number infested	Number parasitized
Windows + Food source	141.8 (3.9)	2.7 (0.8)	1.6 (0.4)
Windows	156.7 (5.5)	3.5 (0.7)	2.3 (0.6)
Food source	155.0 (7.2)	2.3 (0.7)	1.4 (0.5)
Control	160.6 (5.3)	1.1 (0.5)	0.7 (0.3)

Table 14. Means and standard errors of proportion of infestation and parasitism for different rearing cage treatments (2004).

Treatment	Infestation (Proportion)	Parasitism (Proportion)
Windows + Food source	0.02 (0.02)	0.43 (0.10)
Windows	0.02 (0.02)	0.49 (0.09)
Food source	0.02 (0.02)	0.34 (0.10)
Control	0.01 (0.01)	0.26 (0.10)

The standard errors are comparatively large due to the low and variable number of infested stems in a sample. Statistical analysis using multivariate analysis of variance showed that there was a significant difference in the overall model which includes the multiple response variables infestation, parasitism and mortality of the WSS larvae (Wilks' Lambda $F = 2.5$; $P < 0.01$). The multivariate analysis of variance also gives individual analysis of variance output for each response variable. There was a significant difference ($F = 3.8$; $df = 7, 52$; $P < 0.01$) in the variation associated with the amount of infestation (number of infested stems in a sample) among the treatments. The significant interaction ($F = 5.4$; $df = 3$; $P < 0.01$) between the number of stems and treatment was also included in the model. There was also a significant difference ($F = 4.9$; $df = 7, 52$; $P < 0.01$) in the variation associated with the percentage of infestation in the treatments.

There was significant difference ($F = 2.5$; $df = 7, 52$; $P = 0.03$) in the variation associated with the amount of parasitism between the treatments, which could be attributed to the significantly greater infestation in some treatments. However, there was no significant difference ($F = 1.4$; $df = 7, 52$; $P = 0.24$) in the percentage of parasitism for the different treatments.

After collecting samples and removing the rearing cages, the remaining wheat was combine harvested. All postharvest residues were stored in emergence barrels. The number of parasitoids emerging from these barrels at the MSU Horticulture Farm in early summer of 2005 is reported in Table 15. The number of parasitoids obtained was neither large enough nor consistent enough for statistical analysis. This was also due to low levels of infestation by the host WSS.

Parasitoid Mass Rearing (2005): WSS adults emerged inside the mass rearing cages from wheat stubs. Unlike 2004, frequent and consistent flight activity was observed inside the cages for several weeks after WSS emergence began. There were large numbers of infested and parasitized stems inside the cages in 2005. The means and associated standard errors for the number and percent of infested and parasitized stems per sample are reported for the different treatments (Tables 16 and 17).

Table 15. Parasitoids emerged from the wheat stubble in rearing cages (2004).

Cage Treatments	Parasitoids emerging from barrels in MSU Horticulture Farm			
	<i>B. cephi</i>		<i>B. lissogaster</i>	
	Male	Female	Male	Female
Windows+ Food source # 1	4	2	0	0
Windows+ Food source # 2	3	3	0	0
Windows+ Food source # 3	3	4	0	0
Windows # 1	5	8	0	0
Windows # 2	0	1	0	0
Windows # 3	14	15	0	0
Food source # 1	4	7	0	0
Food source # 2	5	6	0	0
Food source # 3	0	1	0	0
Control # 1	4	3	0	0
Control # 2	1	1	0	0
Control # 3	2	7	0	0

Table 16: Infestation and parasitism means for different rearing cage treatments (2005).

Treatment	Number of stems split	Number infested	Number parasitized
Windows + Food source	93.7 (1.9)	49.8 (2.0)	38.0 (1.6)
Windows	91.0 (2.2)	52.0 (2.2)	39.8 (1.9)
Food source	95.1 (2.5)	48.1 (2.3)	36.5 (1.9)
Control	92.9 (2.0)	45.9 (2.2)	36.5 (1.9)

Table 17: Means and standard errors of the proportion of infestation and parasitism for different rearing cage treatments (2005).

Treatment	Infestation (Proportion)	Parasitism (Proportion)
Windows + Food source	0.54 (0.03)	0.77 (0.01)
Windows	0.58 (0.03)	0.77 (0.01)
Food source	0.51 (0.02)	0.76 (0.01)
Control	0.50 (0.02)	0.76 (0.01)

Multivariate analysis of variance showed that there was significant difference in the overall model which includes the multiple response variables, infestation, parasitism, and mortality of the WSS larvae (Wilks' Lambda $F = 2.5$; $P < 0.01$). The multivariate analysis of variance also gives individual analysis of variance output for each response variable. There was a significant difference ($F = 3.5$; $df = 15, 92$; $P < 0.01$) in the variation associated with the amount of infestation (number of infested stems in a sample) among the treatments. There was also a significant difference ($F = 3.5$; $df = 15,$

92; $P < 0.01$) in the variation associated with the percentage of infestation among the treatments.

There was significant difference ($F = 4.7$; $df = 15, 92$; $P < 0.0001$) in the variation associated with the amount of parasitism among the treatments which could be attributed to the significantly greater infestations in some treatments. But, there was also a significant difference ($F = 2.5$; $df = 15, 92$; $P < 0.01$) in the variation associated with the percentage of parasitism for the different treatments. The significant interaction between the number of stems and treatment ($F = 3.9$; $df = 3$; $P = 0.01$) was also included in the model.

After collecting samples and removing the rearing cages, the remaining wheat was combine harvested. All postharvest residues were stored in emergence barrels. The number of parasitoids emerging from these barrels at the MSU Horticulture Farm in early summer of 2006 is reported in Table 18. The data were analyzed for treatment differences in the number of parasitoids emerged and sex ratio. The emergence started from 31 May and lasted until 12 July, 2006.

Multivariate analysis of variance (SAS, 1990) showed that there was no significant difference in the overall model which includes the multiple response variables, number of male and female *B. cephi* and *B. lissogaster* (Wilks' Lambda $F = 2.6$; $P > 0.05$). The multivariate analysis of variance also gives individual analysis of variance output for each response variable. There was no significant difference in the number of male ($F = 0.8$; $df = 3, 8$; $P = 0.52$) or female ($F = 0.5$; $df = 3, 8$; $P = 0.73$) *B. cephi* among the treatments. There was significant difference ($F = 4.8$; $df = 3, 8$; $P = 0.04$) in the number of male *B. lissogaster*, while there was no significant difference ($F = 4.0$; $df = 3,$

8; $P > 0.05$) in the number of females of this species among the treatments. Even though statistical analysis shows that the number of *B. lissogaster* males was significantly different among the treatments; the difference would not be biologically significant due to low emergence numbers for this species.

Table 18. *B. cephi* and *B. lissogaster* parasitoids emerging from the barrels at the MSU Horticulture Farm (2006). Parasitoids are emerging from the wheat residues obtained from the mass rearing cages in 2005.

Treatments	<i>B. cephi</i>		<i>B. lissogaster</i>	
	Male	Female	Male	Female
Windows+ Food source # 1	123	66	5	0
Windows+ Food source # 2	69	13	6	0
Windows+ Food source # 3	57	28	3	0
Food source # 1	110	35	3	0
Food source # 2	75	30	3	0
Food source # 3	39	15	1	0
Windows # 1	57	27	11	0
Windows # 2	79	38	6	0
Windows # 3	103	42	8	0
Control # 1	156	64	11	0
Control # 2	65	29	5	1
Control # 3	128	37	7	1

WSS Infestation Variation as Influenced
by the Method of Introduction (Objective 2)

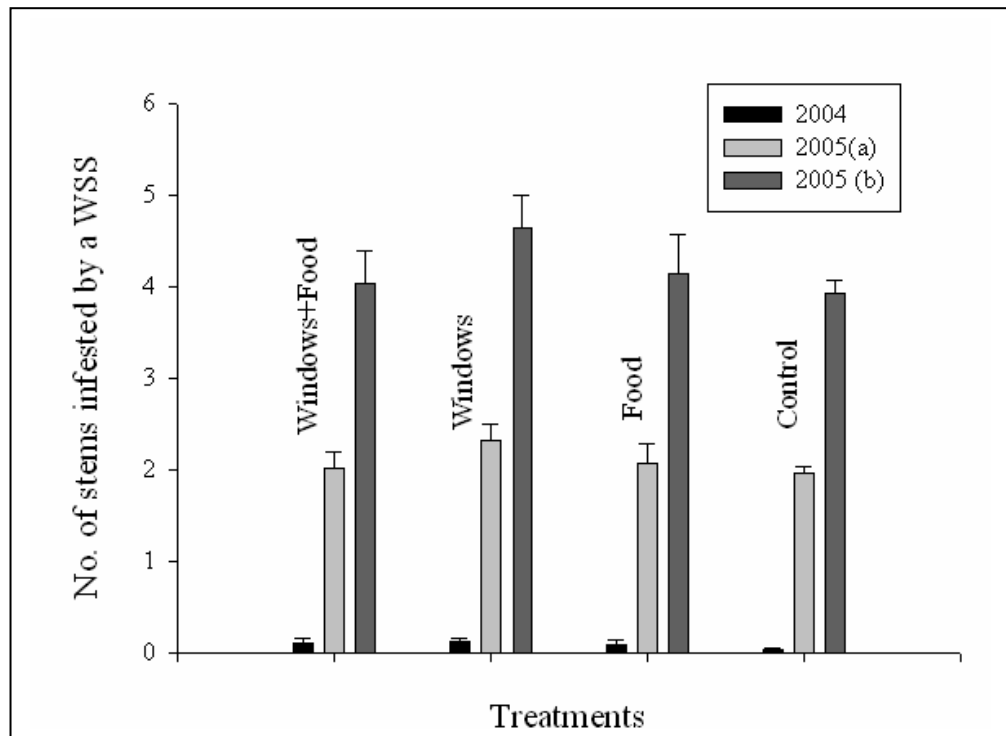
The disappointing levels of infestation in the 2004 experiment was the basis for the allowing the WSS to emerge inside the rearing cages from stubs in 2005. This was not ideal because the exact number of infesting females could not be recorded. However, unlike in 2004, frequent and consistent flight activity was observed inside the cages for several weeks after emergence began. There were large numbers of infested stems with parasitized larvae inside the cages in 2005. The information on the number of infested stems from the total samples collected from a mass rearing cage was extrapolated for 1000 wheat stems. This was used to estimate the number of wheat stems a WSS adult would infest in each treatment cage for the two seasons. The number of stems in the total samples for a cage ranged from 800 to 950.

The mean number of infested stems per WSS female in 2004 is given in Table 19, along with the calculated mean number of infested stems per female in 2005, assuming a 50:50 sex ratio. The numbers are very different for the two years, but to be conservative, we have also calculated the mean number of infested stem per female, assuming that every emerged adult WSS was female (Table 19). The number of infested stems per female is still quite large, showing that the effect is probably due to the reduced handling of the adults leading to much greater success at oviposition (Figure 12).

Table 19. Means and standard errors of number of wheat stems infested by a WSS female. 2004: the mean number of infested stems per WSS female. 2005(a): the mean number of infested stem per female assuming that every emerged adult WSS was female. 2005(b): the mean number of infested stems per female assuming a 50:50 sex ratio.

Treatments	2004	2005(a)	2005(a)
Windows + Food source	0.1 (0.1)	2.0 (0.2)	4.0 (0.4)
Food source	0.1 (0.1)	2.1 (0.2)	4.1 (0.4)
Windows	0.1 (0.0)	2.3 (0.2)	4.7 (0.4)
Control	0.0 (0.0)	1.9 (0.1)	3.9 (0.2)

Figure 12. Bar diagram showing the number of wheat stems infested by a WSS female (2004 -05). Here, Year 1 gives the mean number of infested stems per WSS female in 2004; Year 2 (a) gives the mean number of infested stem per female in 2005, assuming that every emerged adult WSS was female; and Year 2 (b) gives the mean number of infested stems per female in 2005, assuming a 50:50 sex ratio.



High Temperature Mortality Studies (Objective 3)

Temperature Variation in the Wheat Canopy

This experiment was conducted to determine if the high temperatures experienced during the summer could cause mortality in WSS larvae, and thus potentially impact the parasitoids. An unparasitized WSS larva is capable of moving inside the stem to avoid high temperatures in hollow stem wheat, while a parasitized larva is paralyzed and unable to move. This makes the study of temperature variation throughout the wheat canopy important.

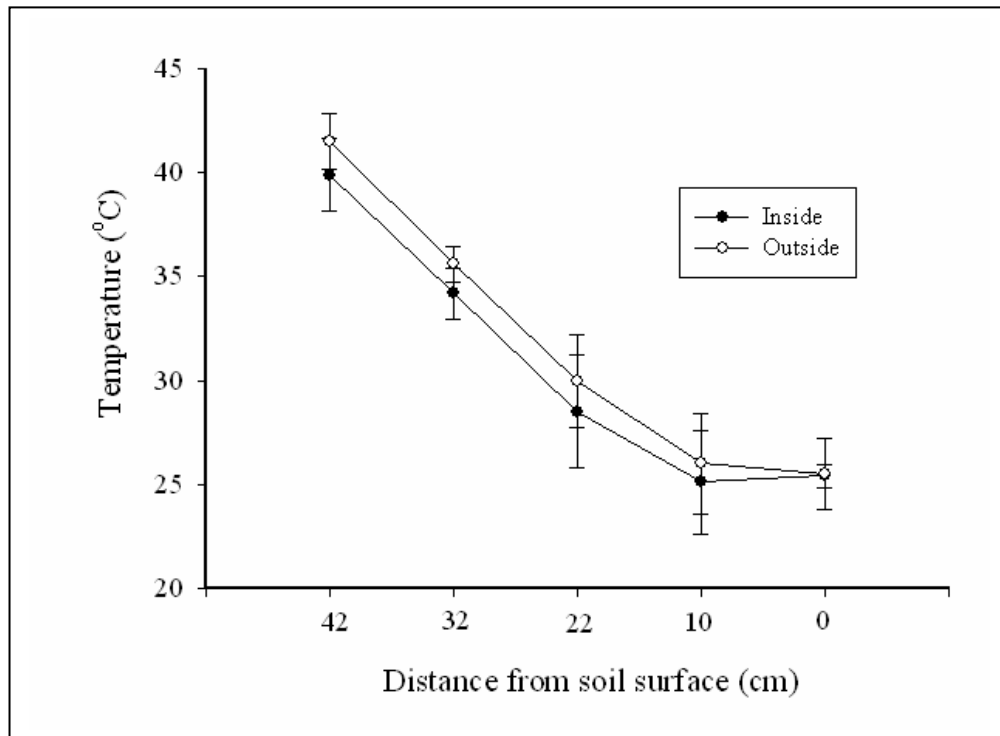
Table 20. Means and associated standard errors for temperature variation ($^{\circ}\text{C}$) at different heights (cm) along the interior and exterior of a wheat stem at a canopy temperature of 47°C . This experiment was conducted in the greenhouse.

Height of measurement	Temperature inside the stem	Temperature outside the stem
42	39.9 (1.8)	41.5 (1.3)
32	34.2 (1.2)	35.6 (0.8)
22	28.5 (2.7)	30.0 (2.2)
10	25.1 (2.5)	26.0 (2.4)
0	25.4 (0.6)	25.5 (1.7)

Potential differences in temperatures between the inside and outside of a wheat stem were measured at different heights above the soil surface in an artificially created canopy temperature of 47°C . The mean and the standard error of the temperature readings obtained are given in Table 20. There was a significant difference ($F = 89.0$; df

= 5, 34; $P < 0.01$) between the inside and outside temperature in the wheat stem as a function of height. The temperature variation at the different heights is plotted in Figure 13.

Figure 13. Means and associated standard errors for temperature variation ($^{\circ}\text{C}$) at different heights (cm) along the interior and exterior of a wheat stem at a canopy temperature of 47°C . This experiment was conducted in the greenhouse.



Potential WSS Larval Mortality in Stems at High Temperatures in the Greenhouse

This experiment was conducted to determine if the unparasitized WSS larvae are able to migrate to the lower regions of the wheat stem to avoid critically high temperatures. The eight wheat stems that were infested with WSS and exposed to high temperature regimes were dissected in the laboratory when the experiment was finished.

Six of the 4 week-old larvae were alive and appeared healthy at the base of the stem. Two larvae had died and one cadaver was located at the base of the stem near the soil surface while the other cadaver was located at a height of 10 cm above the soil surface. The average temperatures varied from 40.4 °C at the top of the stem to 25.5 °C at the base of the stem, which is a similar temperature range to that reported in the previous experiment.

Temperature-induced Mortality of Overwintered WSS in Stubs in the Greenhouse

Rationale for Using WSS Larvae in Diapause: The air temperature used for the experiment averaged 29 °C and could occur naturally for this development stage of WSS. The temperature variations at different points on the exterior of the stub are plotted in Figure 14. As might be expected, there are significant differences in the temperatures ($F = 121.5$; $df = 2, 57$; $P < 0.01$) between the three different locations on the exterior of the stubs which are in contact with the soil. The means and standard errors for the temperatures are given in Table 21. Even at a moderate air temperature, the soil surface temperatures in this experimental layer of soil reach levels that could cause mortality of the overwintering WSS larvae.

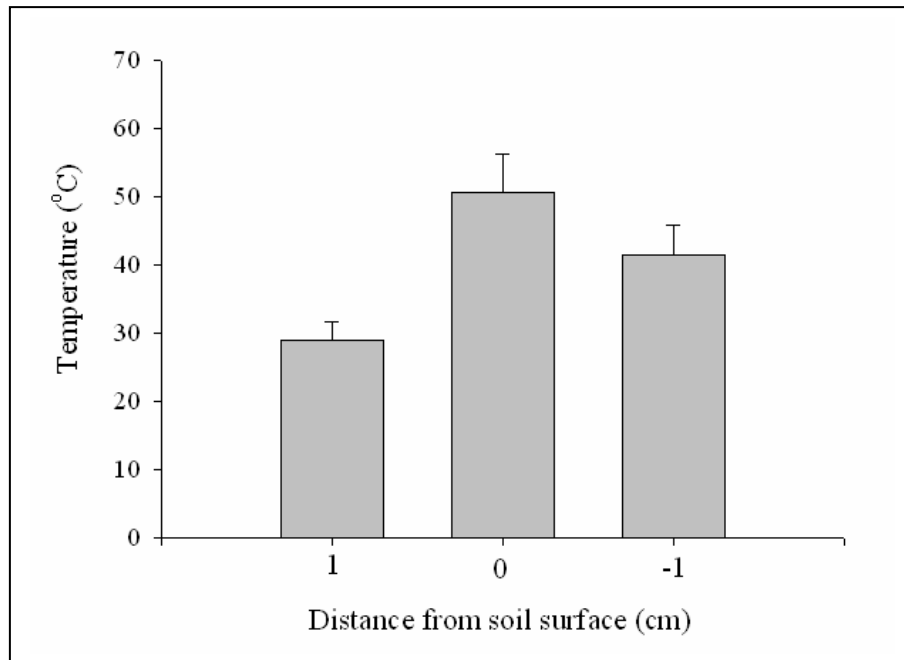
Out of the 60 stubs used in this experiment, 21 contained dead larvae. The surviving larvae remained very active 24 hr after the experiment. Most of these had emerged from the hibernaculum, probably so that they could move along the length of the stub to avoid the highest temperatures. The average temperature varied from 29 °C to 50.6 °C on the exterior and along the length of the stub, with the highest temperature being close to the soil surface. In the unheated control, four WSS larvae were found

dead, out of the 30 stubs that were split. However, two out of the 21 dead larvae in the heated treatment, and two of the four dead larvae in the unheated control had fungal growth on the cadavers, indicating they had obviously died from other causes.

Table 21. Means and associated standard errors for temperature variation ($^{\circ}\text{C}$) at specific locations on the exterior of the stubs at an air temperature of 29°C . The stubs contain overwintering WSS larvae and the experiment was conducted in the greenhouse.

Location	Temperature
Above the soil (1cm)	29.0 (0.6)
Soil Surface	50.6 (1.3)
Below the soil (1 cm)	41.6 (1.0)

Figure 14. Means and associated standard errors for temperature variation ($^{\circ}\text{C}$) at specific locations on the exterior of the stubs at an air temperature of 29°C . The stubs contain overwintering WSS larvae and the experiment was conducted in the greenhouse.



Overall, these data show that temperature-induced mortality of diapausing larval WSS may occur at biologically relevant temperatures.

Pupae: Experiments were conducted using WSS pupae at high and moderate ambient air temperatures. The experiment was similar except that the temperature measurements were taken inside the stub. For the high temperature exposure at 39 °C ambient, the variation in temperature at different locations on the interior of the stub is plotted in Figure 15. As expected, there is a significant difference ($F = 317$; $df = 2, 75$; $P < 0.01$) in temperatures between the different locations and the mean temperatures and the associated standard errors are given in Table 22.

Figure 15. Means and associated standard errors for temperature variation (°C) at specific locations on the interior of the stubs at an air temperature of 39 °C. The stubs contain WSS pupae and the experiment was conducted in the greenhouse.

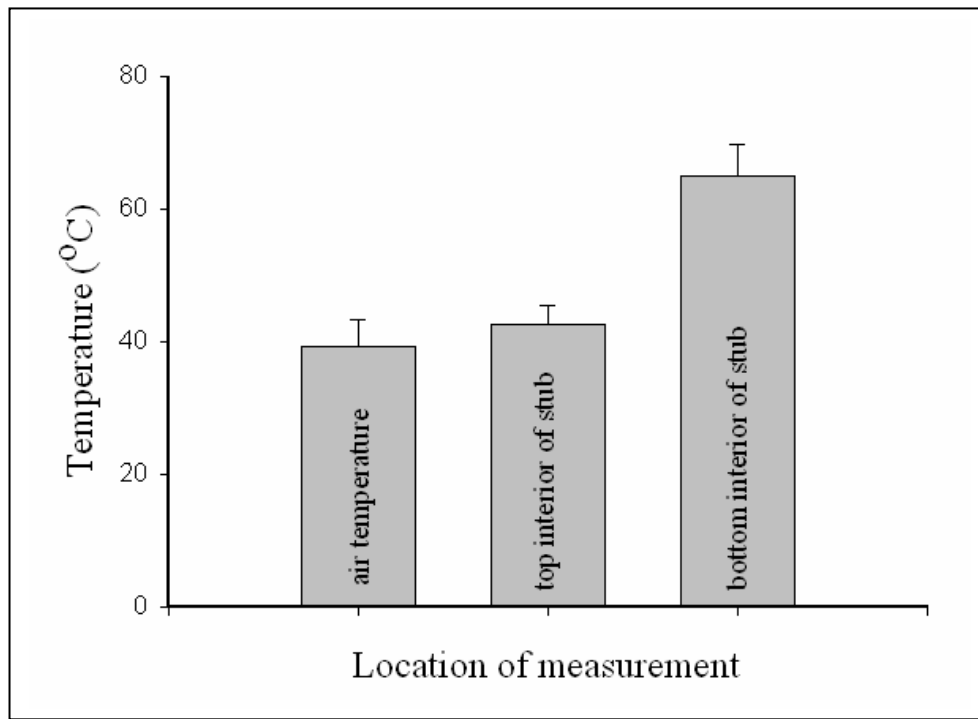


Table 22. Means and associated standard errors for temperature variation ($^{\circ}\text{C}$) at specific locations on the interior of the stubs at an air temperature of 39°C . The stubs contain WSS pupae and the experiment was conducted in the greenhouse.

Location	Temperature
Top of cut stem	42.6 (2.9)
Bottom of cut stem	65.0 (4.7)

Out of the 50 WSS-cut wheat stems of the first replication, 26 pupae were found dead, while 32 were found dead in the second. The average temperature varied from 39 to 65°C along the interior of the WSS-cut stem length, the highest interior temperature being at the bottom of the cut stem which is in contact with the soil. In the control, five out of 25 WSS pupae were found dead for the first replication while the second had four dead pupae. This shows that there is a considerable influence of temperature on the mortality of the pupal stage of the WSS.

For the moderately high temperature exposure at 25°C ambient, the variation in temperatures at the different locations on the interior of the stub is plotted in Figure 16. There is significant difference ($F = 554.7$; $df = 2, 72$; $P < 0.01$) between the locations and the mean temperatures and associated standard errors are given in Table 23.

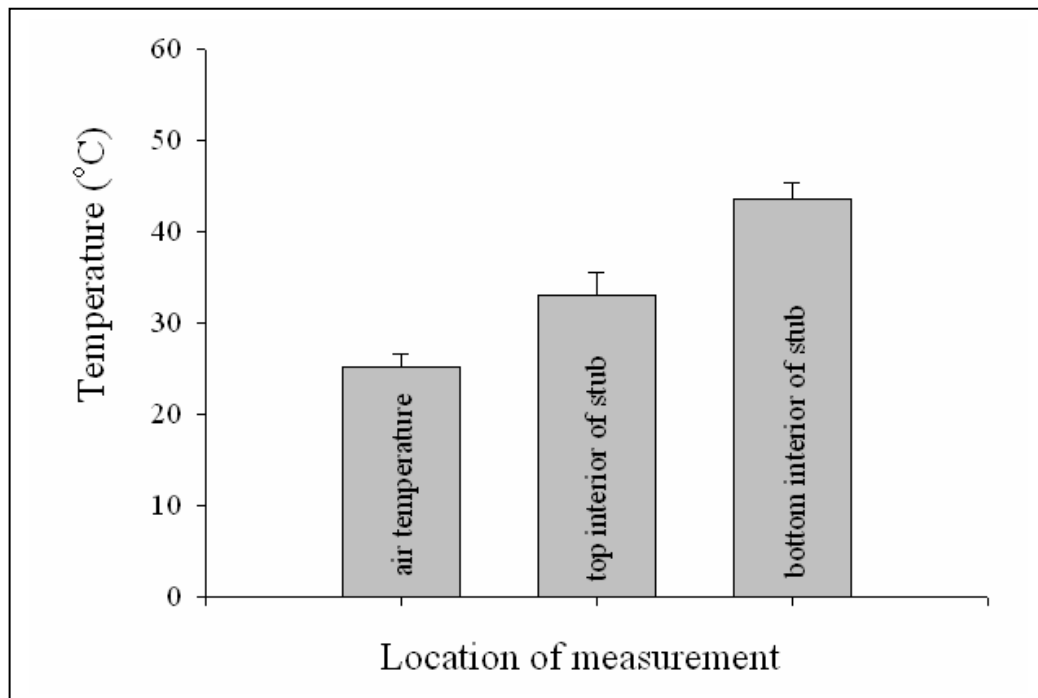
For this moderate temperature experiment the first experimental replicate of 50 stubs contained 23 dead pupae, while 28 were dead in the second replicate of 50 stubs. The average temperature varied from 25°C to 44°C on the interior and along the length of the stub with the highest temperature being recorded at the bottom of the stub embedded in the heated soil. In the unheated control, two out of 25 WSS pupae were

found dead in the first replication and one of 25 was dead in the second replicate. This demonstrates that even moderate air temperature could cause mortality of the pupal stage of WSS at the soil surface.

Table 23. Means and associated standard errors for temperature variation ($^{\circ}\text{C}$) at specific locations on the interior of the stubs at an air temperature of 25°C . The stubs contain WSS pupae and the experiment was conducted in the greenhouse.

Location	Temperature
Top of cut stem	33.1 (2.4)
Bottom of cut stem	43.5 (1.8)

Figure 16. Means and associated standard errors for temperature variation ($^{\circ}\text{C}$) at specific locations on the interior of the stubs at an air temperature of 25°C . The stubs contain WSS pupae and the experiment was conducted in the greenhouse.



Temperature Variation in
the Field at the WSS Pupal Stage

In May, the WSS will pupate in Montana (Morrill et al. 1996). Therefore, on two days, one featuring high temperatures and another when temperatures are more moderate, ambient and several soil position temperature measurements were recorded.

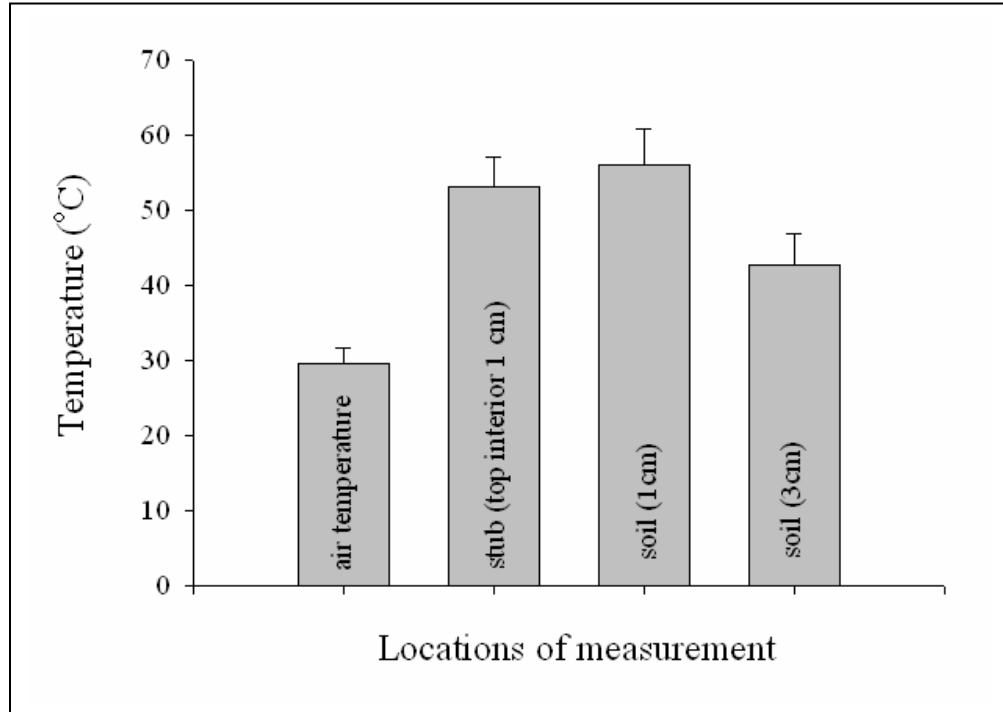
For the hotter day (18 May, 2006), when the average air temperature was 30 °C, the temperature variation at two different soil depths, and one inside the stub at 1 cm depth, were plotted (Figure 17). There were significant differences ($F = 469$; $df = 3, 196$; $P < 0.01$) in the variation in temperature among the four different positions and the mean temperatures and the associated standard errors are given in Table 24.

Table 24. Means and associated standard errors for temperature variation (°C) at a specific location on the interior of the stub, and at two soil depths, when the air temperature is 30 °C. The experiment was conducted in the MSU Post Farm.

	Temperature		
	Mean (SE)	Minimum	Maximum
Inside the stub	53.1 (4.1)	46.1	61.4
Soil (1 cm depth)	56.1 (4.8)	48.3	64.3
Soil (3 cm depth)	42.8 (4.1)	36.0	52.4

These results confirm the high temperature field exposure of the pupal stage of WSS is likely. Even when the air temperature average is only 30 °C, the soil temperature at a 1 cm depth averaged 56 °C which is a critical temperature for sawfly pupae, as will be presented below. The temperature was lower at greater soil depths. The temperature in the stub interior at a 1cm depth was close to the soil temperature at the surface.

Figure 17: Means and associated standard errors for temperature variation ($^{\circ}\text{C}$) at a specific location on the interior of the stub, and at two soil depths, when the air temperature is 30°C . The experiment was conducted in the MSU Post Farm.



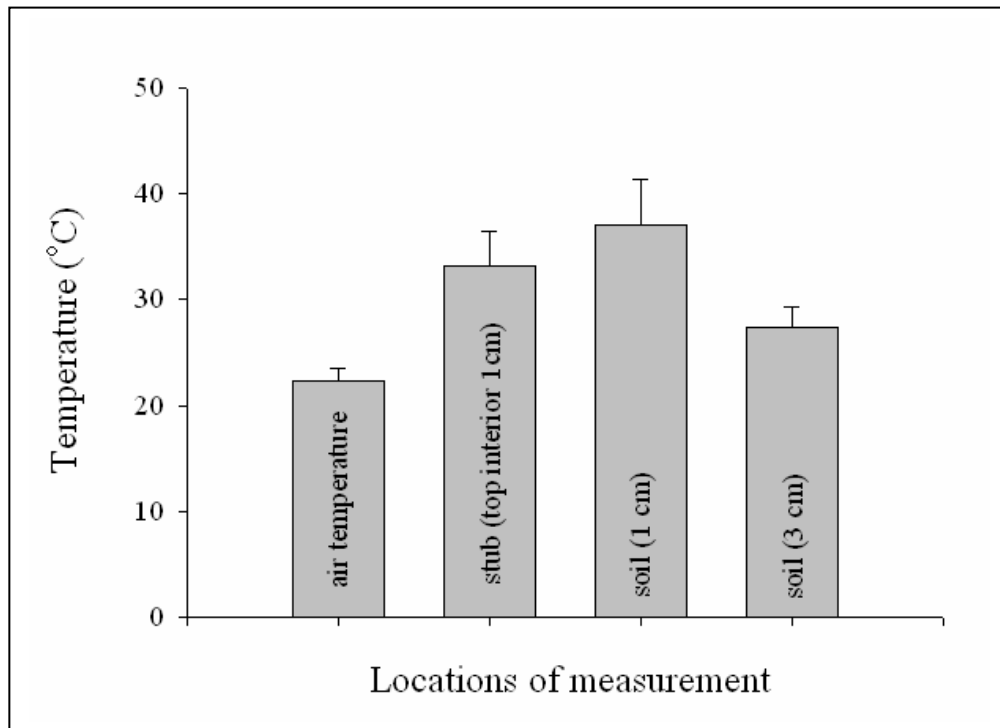
For the moderate temperature day (24 May, 2006), the variation in temperature at different locations on the stub surface is plotted in Figure 18. There were significant differences ($F = 250.9$; $df = 3, 196$; $P < 0.01$) among the four locations and the temperature means and the associated standard errors are given in Table 25.

Even when the average air temperature was 22°C , the average soil temperature at the 1 cm depth reached as high as 38°C . The peak temperature observed for the soil surface at the 1 cm depth was 45°C , which could cause mortality of WSS pupae. The temperatures were lower, deeper in the soil and the temperatures inside the stubs were similar to the soil temperature at the 1 cm depth.

Table 25. Means and associated standard errors for temperature variation ($^{\circ}\text{C}$) at a specific location on the interior of the stub, and at two soil depths, when the air temperature is 22°C . The experiment was conducted in the MSU Post Farm.

	Temperature		
	Mean (SE)	Minimum	Maximum
Inside the stub	33.3 (3.2)	27.7	39.6
Soil (1 cm depth)	37.1 (4.3)	29.5	44.5
Soil (3 cm depth)	27.4 (2.0)	24.7	33.0

Figure 18: Means and associated standard errors for temperature variation ($^{\circ}\text{C}$) at a specific location on the interior of the stub, and at two soil depths, when the air temperature is 22°C . The experiment was conducted in the MSU Post Farm.



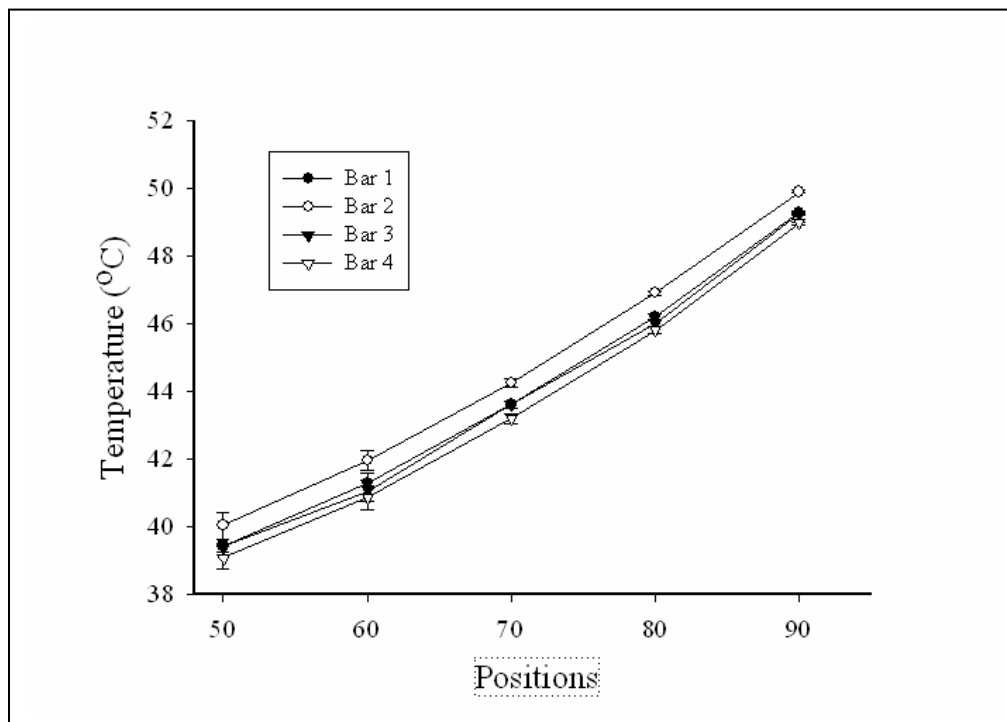
An experimental complication that was also observed was that there was some variation in temperatures due to soil disturbance, and the soil needed to be loosened to

acquire the temperature data. Thus, undisturbed soil probably had lower temperatures than the soil that was loosened to take the measurements at the 3 cm depth (data unavailable).

Laboratory: LTe₅₀ of WSS Larvae and Pupae

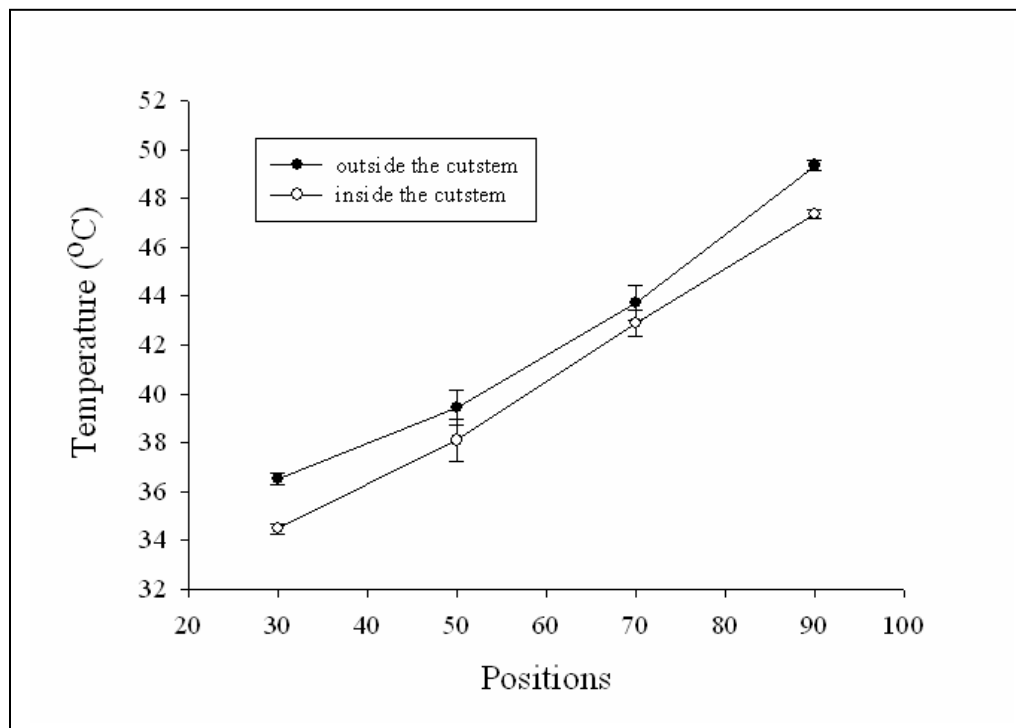
Diapausing larvae: The temperature the insects were exposed to on each of the four different bars for the five different positions is shown in Figure 19. Although there was some low variability in temperatures across the bars, this will not impact the estimation of lethal temperatures and times.

Figure 19. Temperature along the four bars of the thermal gradient equipment at five different positions.



The temperature in the stub interior and the stub exterior for four positions along the bar are shown in Figure 20. The interior of the stub is always a little cooler than the exterior of the stub, and therefore, the lethal temperature calculations will be conservative given that the bar temperature is slightly greater than the temperature experienced by the immature WSS.

Figure 20. Temperature inside and outside a wheat stub on the thermal gradient equipment at four different positions.



The temperature-induced mortality curves observed for diapausing WSS larvae were sigmoidal. All of the data obtained provided reasonable estimates of LT_{50} and LT_{95} values, although the data did not fit as well for the shorter duration bioassays

(Table 26). In Table 26, plots of the backtransformed, fitted mortality lines are referred to. When plotted against the original mortality data that is also shown in Figure 21.

Table 26. The lethal temperature values for the WSS larvae at different time intervals (Figure 21)

Time (h)	Parameter	Value(°C)	95% Confidence Limits (°C)	Pearson χ^2	Significance $P > 0.05$
2	LT _{e50}	47.4	(46.7, 48.3)	56.0	0.89
	LT _{e95}	52.7	(51.3, 55.0)		
3	LT _{e50}	44.3	(43.6, 45.2)	53.6	0.93
	LT _{e95}	50.6	(49.1, 52.8)		
4	LT _{e50}	43.8	(43.2, 44.4)	51.1	0.96
	LT _{e95}	48.1	(47.0, 49.7)		
5	LT _{e50}	42.0	(41.5, 42.5)	50.0	0.97
	LT _{e95}	45.2	(44.4, 46.5)		

Pupal Stage: The temperature-induced mortality pattern observed for WSS pupae was collected as per the larvae. These data also showed a sigmoidal mortality pattern and the parameters and backtransformed mortality lines are listed in Table 27. An obvious difference between the calculated values for the larvae and the pupae are the decreasing lethal temperature requirements for the larvae in longer bioassays, while pupal mortality is fairly constant across the bioassays of different duration Table 27. A key factor in this is that increased larval activity may enhance mortality compared to the quiescent pupae.

Figure 21. Temperature induced mortality in WSS post diapausing larvae and pupae at different time intervals

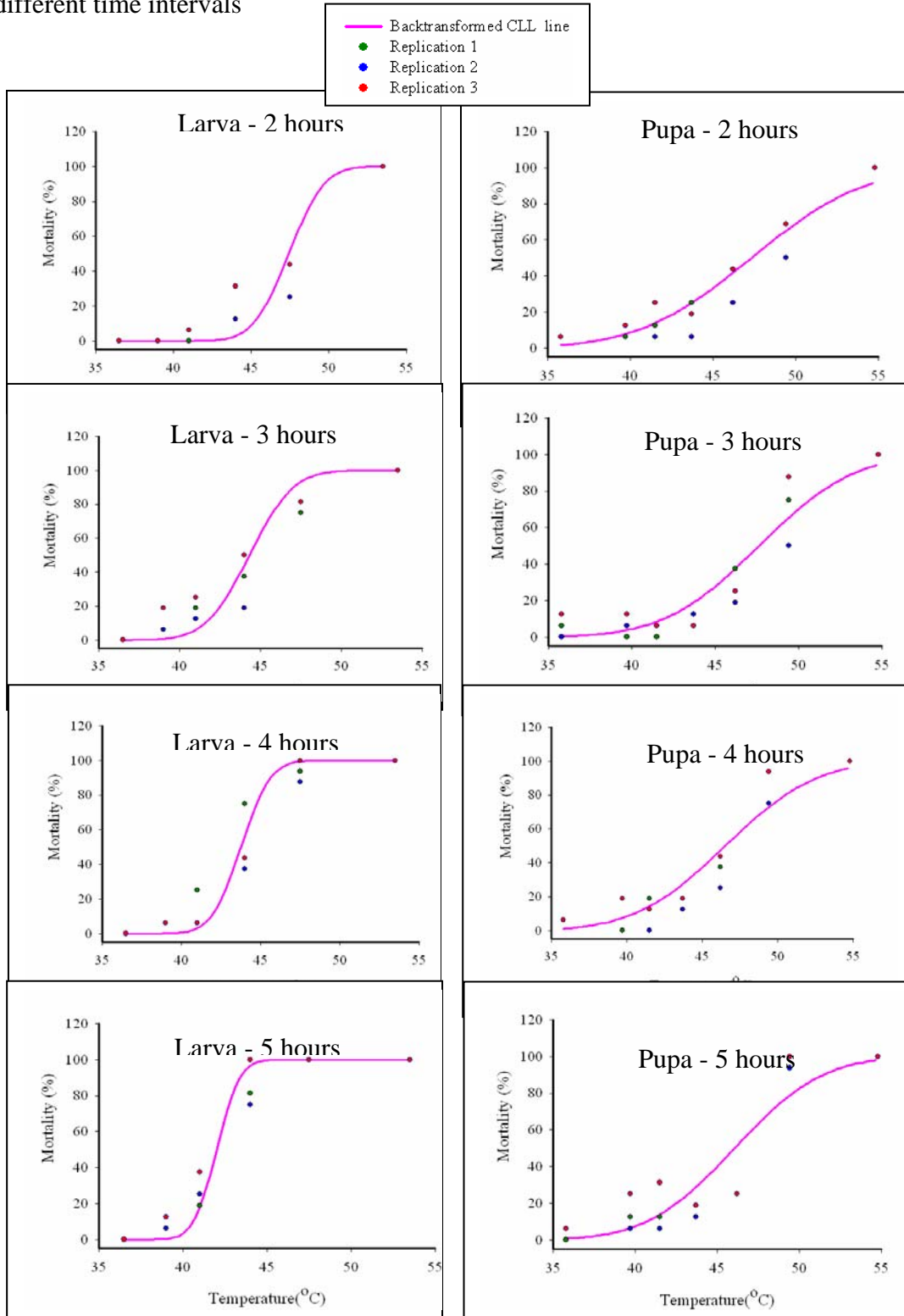


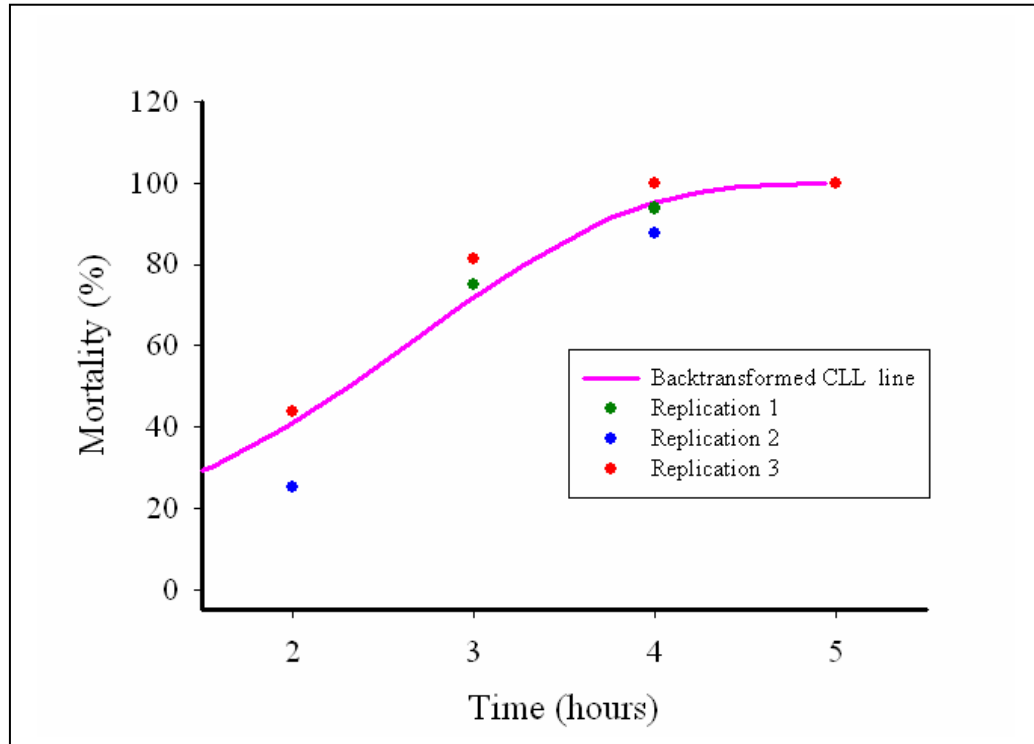
Table 27. The lethal temperature values for the WSS pupae at different time intervals (Figure 21)

Time (h)	Parameter	Value (°C)	95% Confidence Limits (°C)	Pearson χ^2	Significance $P > 0.05$
2	LT _{e50}	47.3	(46.3, 48.6)	102.8	0.06
	LT _{e95}	56.2	(54.0, 59.4)		
3	LT _{e50}	47.7	(46.2, 49.6)	251.5	<0.01
	LT _{e95}	55.0	(52.4, 59.8)		
4	LT _{e50}	46.5	(45.6, 47.7)	112.3	0.02
	LT _{e95}	54.3	(52.4, 57.2)		
5	LT _{e50}	46.1	(45.1, 47.2)	133.2	<0.01
	LT _{e95}	53.0	(51.1, 55.9)		

Laboratory: LT₅₀ of Sawfly Larvae and Pupa

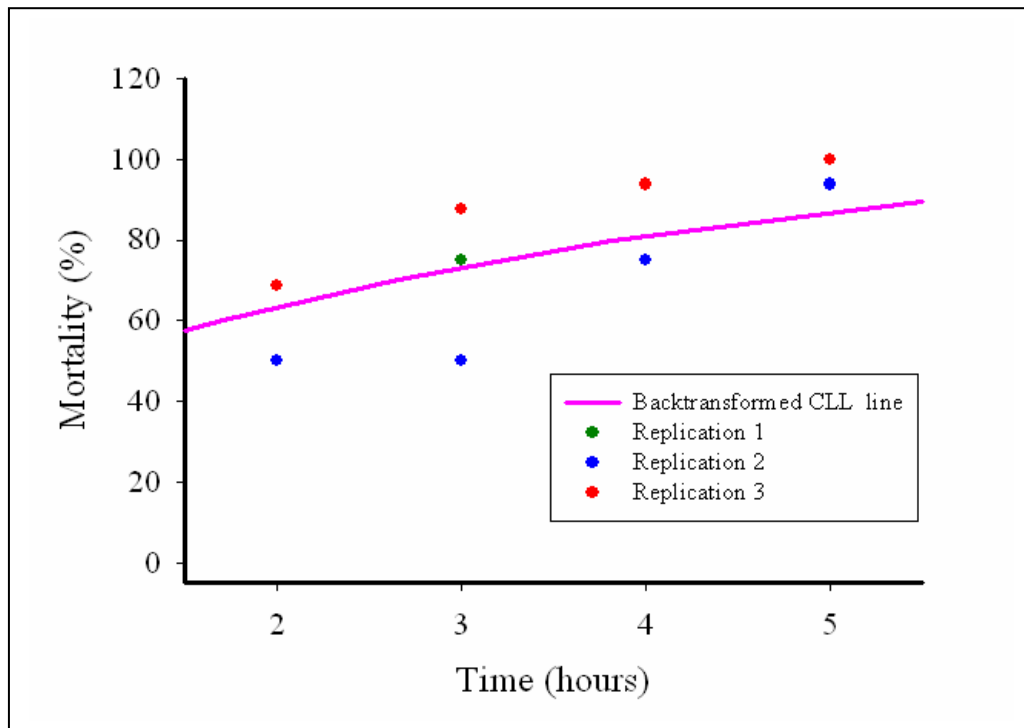
The mortality data for the four different time intervals (2, 3, 4, and 5 hr) were obtained from separate experiments, so it was possible to collect lethal time values at a constant temperature as well. For the diapausing WSS larvae, the mortality pattern at the selected temperature of 48 °C was again sigmoidal. The LT₅₀ is the lethal time for mortality in 50% of the population at a constant temperature. The LT₅₀ value was 2.2 hr, with 95% confidence limits ranging from 1.8 to 2.4 hr. The LT₉₅ is the lethal time for mortality in 95% of the population at the same constant temperature. The LT₉₅ value was 3.9 hr with 95% confidence limits ranging from 3.5 to 4.7 hr. The Pearson χ^2 was 45.3 with a probability of a greater value of χ^2 at 0.50. The backtransformed mortality line and the original data are plotted in Figure 22.

Figure 22. Temperature induced mortality over time in WSS post diapausing larvae at 48 °C.



Lethal times for WSS pupae were calculated at a constant temperature of 49 °C. The LT_{50} value was 1.6 hr, with 95% confidence limits ranging from 0.6 to 2.3 hr. The LT_{95} value was 5.4 hr, with 95% confidence limits ranging from 4.4 to 9 hr. The Pearson χ^2 was 66.4 with a probability of a greater value of χ^2 at 0.03. The backtransformed mortality line and the original data are plotted in Figure 23. For the diapausing WSS pupae at 49 °C, the mortality pattern observed over time was linear. The lethal time values of the two immature stages cannot be directly compared because they were calculated at different temperatures, but they are quite similar for the two nearly identical test temperatures.

Figure 23. Temperature induced mortality over time in WSS pupa at 49 °C



CHAPTER FOUR

DISCUSSION

Mass Rearing of *B. cephi* and *B. lissogaster*

The greenhouse rearing cage experiments provided preliminary indications that the percentage of infestation was higher in cages with sufficient visible light and might be an important factor affecting WSS infestation and hence parasitism. The influence of other factors on WSS infestation could not be determined, due to low levels of infestation for these experiments in both 2004 and 2005. Even though a small amount of parasitism was seen in the cages with supplemented visible light, this illustrated little beyond proof of concept. The indoor and outdoor experiments conducted in 2004 and 2005 with the small cages should be repeated to confirm the importance of visible light in greenhouse rearing and also to assess the importance of the other factors, using the current infestation procedures used in the laboratory.

When we measured the temperature, ultraviolet, and visible light readings at the mass rearing cage locations in the field, these were significantly lower ($P < 0.01$) inside the cages than outside. The interior of the cages with windows had significantly higher ultraviolet ($P < 0.01$) and visible light intensity ($P < 0.01$) than the cages without windows. The temperatures in the cages with windows was also higher, but was only significantly so ($P < 0.01$) when the readings inside the cages were analyzed separately. These temperatures were still lower than the actual field temperature, so the cages are likely to increase the percent survival of the host WSS larvae and hence the parasitoids.

The temperature mortality studies helped us to identify the critical temperatures for WSS larvae with restricted movement.

In the initial experiments for cage window material selection, only a small difference was observed for the amount of visible light transmitted by the window material and the cage material (Figure 11), when the readings were taken at a distance of 10 cm from the source. But when visible light measurements were taken inside the rearing cages with and without windows, there was a 30% increase in visible light in the cages with windows. The two experiments showed different results due to the effect of the angle of incidence of visible light at varying distances.

The mass rearing experiment in the field in 2004 showed that there is an effect of windows on the amount and percentage of infestation, and on the amount of parasitism in the rearing cages. However, there was no significant difference in the percent parasitism between the treatments. But, the overall objective of these experiments, which was to mass rear the parasitoids of WSS, was not accomplished in 2004. We obtained low levels of infestation which limited the potential parasitism. The percentage of parasitized larvae was still quite high for the first season, which indicates that the impediment for parasitoid mass rearing was obtaining sufficient infestation in wheat by the WSS adults. The reduced flight activity of the adults in the rearing cages supported that there was a critical flaw in the method of introduction of WSS.

The experiment for the second season was modified based on the above observations. We obtained a much greater level of infestation, which could be attributed to the method of release of WSS into the cages. This improvement was more dramatic than the subtle treatment effects that we also observed. However, both wheat stem

infestation and the resulting parasitism were significantly increased by the increased light availability provided by the windows in 2004 and in 2005. This shows that the light is important to infestation by WSS. There was also an increase in the percentage of parasitism for the cages with windows in 2005 which emphasizes the significance of either ultraviolet or visible light for parasitoids. The higher amount of ultraviolet radiation in the windowed cages could benefit the sawflies and possibly the parasitoids by helping in the slow oxidation of cuticular hydrocarbons and diacetates for the production of pheromones (Bartelt et al. 2002). This could influence the mating and the production of female progeny. Therefore, further studies should be conducted to determine the importance of ultraviolet light in mass production, because the ultraviolet transmitting materials are three times more costly when compared to the materials which allow only visible light. The presence or absence of flowering plants and honey vials as food source for the parasitoids was not found to have any influence on the mass production of parasitoids in these experiments. However, the numbers of parasitoids introduced were quite large, and the effects of supplemental food might be more beneficial at lower parasitoid densities. There was no significant difference between the treatments for the mortality of WSS larvae, or for the emergence of the first generation of these larval parasitoids, for either season.

The high percent of parasitism for both years also suggests that lowering the number of parasitoids introduced into the cages could still provide sufficient parasitism for the mass production. The occurrence of large number of parasitoids in the rearing cages also could have resulted in a greater time allocation for host seeking than food searching due to severe competition. From different scientific studies it is evident that

parasitoid longevity is increased in the presence of food sources like flowering plants and honey solution (Stapel 1997, Gourdine et al. 2005, Sisterson et al. 2002). However, if there are large numbers of parasitoids in the cage, this increase in longevity would not influence the amount of parasitism. Therefore, further studies should be conducted by introducing fewer parasitoids into rearing cages with treatment factors like food sources, and variable amounts of visible and ultraviolet light.

The male-to-female sex ratio of the emerging parasitoids from the 2005 mass rearing experiment was found to be unusually high. This might have occurred due to a reduced level of mating inside the rearing cages. A large number of female parasitoids were introduced into the cages with few or no male parasitoids, assuming that the females would have already mated in the field and, also there were no males available from the field at the time these parasitoids were collected. Since these hymenopteran parasitoids are haplodiploid, the absence of mating would result in male progeny (Flanders 1946, Cook 1993). There is also a possibility that the introduced females were mated in the field, and due to differing conditions in the cage environment were unable to utilize the sperm or produce diploid female progeny. However, a low number of female parasitoids were also obtained from the emergence barrels which indicate that some of the introduced females had mated in either the field or the cages. Therefore, further studies should be conducted to find the optimum sex ratio of the parasitoids that is required to produce the greatest number of female parasitoids. If there is sufficient number of males in the cages providing a readily available sperm supply for the females, it could result in the production of large numbers of first generation female parasitoids which might ultimately influence the second generation parasitoid numbers. While dissecting the

samples of the 2005 mass rearing experiment, we came across large numbers of emergence holes which was probably made by the first generation parasitoids. It is possible that these were male progeny which were unable to parasitize the WSS larvae in the cages and eventually perished. Further studies should also be conducted to learn if the mating status of the female parasitoids could influence its foraging ability.

The number of WSS females introduced into the mass rearing cages is also significant. Smaller numbers would result in insufficient hosts for parasitism, while larger numbers could result in parasitoid larval mortality from the cannibalism reported among WSS larvae. A paralyzed and parasitized WSS larva would be an easier target of cannibalism when compared to an actively feeding larva (Weaver et al. 2005). Therefore, future studies are required to find the lower optimum numbers and ratio of the WSS adults and parasitoids to be introduced into a rearing cage.

High Temperature Mortality Studies

Several studies indicated that high temperatures could be one of the mortality factors affecting WSS immatures in the field. The experiment measuring inside and outside temperatures of the wheat plant showed that stem interiors were cooler, which suggest that larvae in growing stems are somewhat protected from the most extreme temperatures. Temperature varied along the length of the growing stem in the canopy, with the coolest temperatures occurring near the soil surface. Even at an artificially induced canopy temperature of 47 °C, significantly lower temperatures occurred near the soil surface. This shows that an active larva exposed to high temperatures might be able

to migrate to lower parts of the wheat stem to survive. This suggests that survival could depend on the behavior of active WSS larvae when exposed to high temperatures.

The results from the mortality experiments in the greenhouse confirm that four week old WSS larvae moved down the frass-filled, hollow wheat stems when exposed to the artificial wheat canopy temperature of 47°C. The high survival rate shows that they were able to behaviorally avoid the highest temperatures. These mature larvae had already bored through the stem nodes which could have aided in the rapid downward movement. This instar is probably most exposed to high temperature in the field. Experiments should be conducted to elucidate the behavior of younger larvae when the wheat canopy is exposed to similar temperatures. Behavior and survival of the younger instars could be limited because they might not be able to migrate as efficiently as the older larvae. Both the absence of previously bored nodes and a slower rate of movement due to smaller body size might reduce survival in younger larvae.

The wheat canopy protects the active instars of the WSS from direct exposure to high temperatures in the growing stems. WSS larvae undergo an obligatory diapause from late summer till next May, when this is broken by the development of the pupal stage. The frass-plugged stub, the hibernaculum, and the insulating layer of soil would be the only protection from direct exposure to widely varying temperatures. Even though the diapausing larvae are primarily exposed to low temperatures in winter, there are days in the spring with elevated temperatures while they are in the diapausing larval or pupal stage. The temperature variations in the soil at different depths, and inside the stub were determined at high and moderate air temperatures in the field. The results confirm the occurrence of high temperatures in field, which are more coincident with the pupal stage

of WSS than the larvae. Even when the average air temperature was 30 °C, the soil temperature average at 1 cm depth was found to surpass the critical temperature that was calculated for WSS pupae. The temperature was lower at greater soil depths. The wheat stub containing the diapausing larval or pupal stage of the WSS could occur at the variable depths below the soil surface in the field was first described by Runyon et al. (2002). This could result in variability in mortality depending on depth and soil condition.

A high rate of mortality was observed when diapausing WSS larvae in stubs were exposed to high temperatures in the greenhouse at a uniform soil depth. This confirms that diapausing larvae in stubs are more vulnerable to high temperature than those in the growing wheat stems, because of the inability to behaviorally escape the unfavorable condition. High mortality was also observed in the WSS pupal stage when exposed to similarly high temperatures. The LT_{e50} for the diapausing WSS larvae decreased by more than 10% as bioassay length increased from two to five hours, while for the pupal stage it decreased by less than 3% for the same increase in bioassay length. The difference may be due to the greater potential for activity and the greater surface area in the larvae.

In addition to assessing potential temperature-induced mortality of the overwintering stages of the WSS, the inactive diapausing larvae and pupae serve as useful models for extreme temperatures that the parasitized and thus inactive WSS host would be exposed in the field. Observations made during the redistribution efforts of overwintered parasitoids at different locations in Montana (Morrill et al. 1998) also suggest that parasitized, paralyzed WSS larvae might be more vulnerable to high

temperatures than unparasitized larvae, especially if healthy larvae can behaviorally avoid extreme heat (Weaver and Morrill, unpublished data).

There are practical limitations in parasitizing WSS larvae under laboratory conditions. Therefore, paralyzed host larvae were not available for the temperature induced mortality studies. It is possible to dissect actively-feeding larvae and then constrain them for exposure to constant high temperatures. However, confining an active feeding stage of the WSS larvae might expose it to other mortality factors like limited food availability and injury arising from possible hyperactivity at high temperatures. The paralyzing toxin from the idiobiont parasitoid also might cause significant changes to the thermal tolerance of the WSS larvae. For these reasons and for convenience, the naturally immobile overwintering larvae and pupae of WSS are more suitable for mortality studies than actively feeding larvae that have been removed from the stem.

The temperature induced mortality studies of the WSS also help to understand the relevance of high temperatures occurring in the field during the mass rearing of parasitoids in the cages at the MSU Post Farm. In the cages, there will be a large number of paralyzed WSS larvae serving as hosts for the developing larval parasitoids. The temperature induced mortality studies allow us to understand the lethal temperatures and exposure times required for host mortality. Therefore, occurrence of comparably lower temperatures inside the rearing cages seems likely to be beneficial for the mass production of the parasitoids.

Summary

A suitable method for the production of a significant amount of WSS infestation and subsequent parasitism has been developed for mass rearing cages. The importance of environmental factors like visible and ultraviolet light, as well as temperature for enhancing the amount of parasitism was also elucidated in this study. The significance of these factors and the role of supplemental food sources for the parasitoids will be better understood when lower densities of parasitoids are introduced into the cages. We require further knowledge on the optimum WSS-parasitoid ratio to be released into the cages, and the optimal sex ratio for the braconids, especially considering the two parasitoid generations. These mass rearing experiments have taken the goal of mass producing a sufficient number of parasitoids for release into the WSS damaged wheat fields of Montana a step closer to practical reality.

REFERENCES CITED

- Ainslie, C. N. (1920). The western grass-stem sawfly, United States Department of Agriculture Bulletin 841.
- Ainslie, C. N. (1929). The western grass-stem sawfly: a pest of small grains. Washington, D.C, United States Department of Agriculture Technical Bulletin 157.
- Bartelt, R. J., A. A. Cosse, 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. *Journal of Chemical Ecology* 28(2): 385-405.
- Blodgett, S. L., and W. T. Lanier (eds.). (1996). Montana State University. Special issue: wheat stem sawfly. *Montana Crop Health Report* 9(11):1-7
- Blodgett, S. L., and W. T. Lanier (eds.). (1997). Special issue: wheat stem sawfly. Montana State University. *Montana Crop Health Report* 9(11):1-8
- Cherian, M. C., and P. S. Narayanaswami (1944). The biology of *Microbracon chilonis*, a larval parasite of *Chilo zonellus* Swin. *Indian Journal of Entomology*. 4: 5-7.
- Church, N. S. (1955). Moisture and diapause in the wheat stem sawfly, *Cephus cinctus* Nort. (Hymenoptera: Cephidae). *The Canadian Entomologist*. 87(2): 84-97
- Clausen, C. P. (1940). *Entomophagous insects*. McGraw-Hill. New York and London.
- Clausen, C. P. (1978). *Introduced parasites and predators of arthropod pests and weeds*. United States Department of Agriculture Handbook. 480. Washington D.C.
- Clarke, A. R., and G. H. Walter (1995). 'Strains' and the classical biological control of insect pests. *Canadian Journal of Zoology*. 73:1777-1790.
- Cook, J. M. (1993). Sex determination in Hymenoptera: a review of models and evidence. *Heredity* 71: 421-435.
- Cosse, A. A., R. J. Bartlet, D. K. Weaver, and B. W. Zilkowski. (2002). Pheromone components of the wheat stem sawfly: identification, electrophysiology and field bioassay. *Journal of Chemical Ecology*. 28(2).
- Criddle, N. (1911). Injurious insects of 1910 at Treesbank, Manitoba. *Journal of Economic Entomology*. 4: 236-241.
- Criddle, N. (1913). Insect pests of southern Manitoba during 1912. *Ontario Entomological Society Annual Report*. 43:97-100.
- Criddle, N. (1915). The Hessian fly and the western wheat stem sawfly in Manitoba,

Saskatchewan, and Alberta. Canada Department of Agriculture Bulletin No.11.

Criddle, N. (1921). Further observations upon the habits of the western wheat stem sawfly in Manitoba and Saskatchewan. *The Agricultural Gazette of Canada* 4(3): 176-177.

Criddle, N. (1922). The western wheat stem sawfly and its control. Canada Department of Agriculture: Pamphlet No.6.

Criddle, N. (1922). The western wheat stem sawfly and its control. Dominion of Canada, Department of Agriculture: Pamphlet 6.

Criddle, N. (1923). The life habits of *Cephus cinctus* Norton in Manitoba. *Canadian Entomologist* 55:1-4.

Criddle, N. (1924). Two problems in natural control. *The Report of the Entomological Society* 33: 16-19.

Davis, E. G., C. Benton and H. W. Somsen (1955). Natural enemies of wheat stem sawfly in North Dakota and Montana. *North Dakota Agricultural Experiment Station, Bimonthly Bulletin* 28(2): 63-65.

Farstad, C. (1940). The development of western wheat stem sawfly, *Cephus cinctus* Norton, in various host plants as an index of resistance. *Iowa State College Journal of Science* 15(1): 67-69.

Farstad, C. W., A. W. Platt, and A. J. McGinnis (1949). Influence of wheat varieties on the sex ratio of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), pp. 27-28. In W.E. Heming [ed.], 80th Annual Report of the Entomological Society of Ontario. Printer to the King, Toronto, Canada.

Farstad, C.W. and L.A. Jacobson (1945). Manual for sawfly workers in Alberta, Canadian Department of Agriculture Science Service, Division of Entomology, Contribution 16.

Feng, M., and R. M. Nowiersky (1992). Spatial distribution and sampling plans for four species of cereal aphids (Homoptera: Aphididae) infesting spring wheat in southwestern Idaho. *Journal of Economic Entomology*. 85: 830-837

Flanders, S. E. (1946). Control of fertilization and sex in Hymenoptera. *Journal of Economic Entomology*. 39: 379-380

Flinn, P. W., and D. W. Hangstrum (2002). Temperature mediated functional response of *Theocolax elegans* (Hymenoptera: Pteromalidae) parasitizing *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in stored wheat. *Journal of Stored Products Research*. 38: 185-190

Godfray, H. C. J., and J. K. Waage (1988). Learning in parasitic wasps. *Nature* 21(2): 331.

- Gourdine, J. S., A. M. Simmons, G. S. McCutcheon, and G. L. Leibee (2005). Floral nectars and honey enhance survival of *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of the diamondback moth (Lepidoptera: Plutellidae). *Journal of Entomological Science*. 40(1): 96-99.
- Holland, J. M., J. N. Perry, and L. Winder (1999). The within-field spatial and temporal distribution of arthropods in winter wheat. *Bulletin of Entomological Research*. 89: 499-513.
- Holmes, N. D., and H. Hurtig (1952). Screening tests of ten contact insecticides on the wheat stem sawfly, *Cephus cinctus* Norton. Canadian Department of Agriculture Science Service Division of Entomology.
- Holmes, N. D. (1954). Ecology of wheat stem sawfly, *Cephus cinctus* (Norton). Thesis, Oregon State College.
- Holmes, N. D., and C. W. Farstad (1956). Effect of field exposure on immature stages of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). *Canadian Journal of Agricultural Science*. 36: 196-202.
- Holmes, N. D., R. L. Larson, L. K. Peterson, and M. D. McDonald (1960). Influence of periodic shading on the length and solidness of the internodes of rescue wheat. *Canadian Journal of Plant Science*. 40: 183-187.
- Holmes, N. D., and L. K. Peterson (1962). Resistance of spring wheats to the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae): 2. resistance to the larva. *The Canadian Entomologist* 94(4): 348-365.
- Holmes, N. D., and L. K. Peterson (1963). Effects of variety and date of seeding spring wheats and location in the field on sex ratio of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae). *Canadian Journal of Zoology*. 41: 1217-1222.
- Holmes, N. D., and L. K. Peterson (1963). Heptachlor as a systemic insecticide against the wheat stem sawfly, *Cephus cinctus* Norton. *The Canadian Entomologist* 95: 792-796.
- Holmes, N. D., W. A. Nelson, L. K. Peterson, and C. W. Farstad (1963). Causes of variations in effectiveness of *Bracon cephi* (Gahan) (Hymenoptera: Braconidae) as a parasite of the wheat stem sawfly. *The Canadian Entomologist* 95(2): 113-126.
- Holmes, N. D. (1975). Effects of moisture, gravity, and light on the behavior of larvae of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae). *The Canadian Entomologist*. 107: 391-401.
- Holmes, N. D. (1977). The effect of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae) on the yield and quality of wheat. *The Canadian Entomologist*. 109: 1591-1598.
- Holmes, N. D. (1978). The wheat stem sawfly. *Proceedings of the Annual Meeting of*

the Entomological Society of Alberta. 2-13.

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

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

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

Korie, S., J. N. Perry, M. A. Mugglestone, S. J. Clark, C. F. G. Thomas, and M. N. Roff (2000). Spatio-temporal associations in beetle and virus count data. Journal of Agricultural, Biological, and Environmental Statistics. 5: 214-239.

Lewis, W. J., E. M. V. Louise, J. H. Tumlinson, J. C. Van Lenteren and D. R. Papaj (1990). Variations in parasitoid foraging behavior: essential element of a sound biological control theory. Environmental Entomology. 19(5): 1183-1193

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. Environmental Entomology. 34(3): 719-726.

Mankin, R. W., D. K. Weaver, M. Grieshop, B. Larson, and W. L. Morrill (2004). Acoustic system for insect detection in plant stems: comparisons of *Cephus cinctus* in wheat and *Metamasius callizona* in bromeliads. Journal of Agricultural and Urban Entomology. 21(4): 239-248 (October 2004)

Marsh, P.M., (1979). Braconidae. In: Catalog of Hymenoptera in America north of Mexico. Smithsonian Institute. Washington D. C.1: 144-294.

McBride, D. K., D. D. Kopp, and C. W. Nyegaard (1989). Wheat stem insect pests and management practices. North Dakota State University Extension Service.

McGinnis, A. J. (1950). Sex ratio studies of the wheat stem sawfly (*Cephus cinctus* Norton). M.S. thesis. Montana State University, Bozeman.

Mendoza, J. P., and D. K. Weaver (2006). Temperature and relative humidity effects on post-diapause larval development and adult emergence in three populations of wheat stem sawfly (Hymenoptera: Cephidae). In Press. Environmental Entomology.

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

Mopper, S., and T. G. Whitham (1992). The plant stress paradox: effects on pinyon sawfly sex ratios and fecundity. Ecology 73: 515-525.

- Morrill, W. L., J. W. Gabor, and G. D. Kushnak (1992). Wheat stem sawfly (Hymenoptera: Cephidae): damage and detection. *Journal of Economic Entomology* 85(6): 2413-2417.
- Morrill, W. L., J. W. Gabor, E. A. Hockett, and G. D. Kushnak (1992). Wheat stem sawfly (Hymenoptera: Cephidae) resistance in winter wheat. *Journal of Economic Entomology* 85(5): 2008-2011.
- Morrill, W. L., J. W. Gabor, and D. Wichman (1993). Mortality of the wheat stem sawfly (Hymenoptera: Cephidae) at low temperatures. *Environmental Entomology* 22(6): 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. *Entomological Society of America*. 87(5): 1373-1376.
- Morrill, W. L. (1995). *Insect pests of small grains*. APS press. St. Paul Minnesota.
- Morrill, W. L., and G. D. Kushnak (1996). Wheat stem sawfly (Hymenoptera: Cephidae) adaptation to winter wheat. *Environmental Entomology* 25(5): 1128-1132.
- Morrill, W. L. (1997). The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), and associated parasitoids in the northern great plains of north america. *Trends in Entomology* 1: 171-174.
- Morrill, W. L. (1998). Parasitism of the wheat stem sawfly (Hymenoptera: Cephidae) in Montana. *Biological control* 12: 159-163.
- Morrill, W. L., and G. D. Kushnak (1999). Planting date influence on the wheat stem sawfly (Hymenoptera: Cephidae) in spring wheat. *Journal of Agricultural and Urban Entomology*. 16:123-128.
- Morrill, W. L., and D. K. Weaver (2000). Host plant quality and male wheat stem sawfly (Hymenoptera: Cephidae) fitness. *Journal of Entomological Science* 35(4): 478-482.
- Morrill, W. L., J. W. Gabor, D. K. Weaver, G. D. Kushnak, and N. J. Irish (2000). Effect of host plant quality on the sex ratio and fitness of female wheat stem sawflies (Hymenoptera: Cephidae). *Environmental Entomology*. 29(2): 195- 199.
- Morrill, W. L., D. K. Weaver, N. J. Irish and W. F. Barr (2001). *Phyllobaenus dubius* (Wolcott) (Coleoptera: Cleridae), a new record of a predator of the wheat stem sawfly (Hymenoptera: Cephidae). *Journal of Kansas Entomological Society* 74(2): 57-59.
- Morrill, W. L., D. K. Weaver, and G. D. Johnson (2001). Trap strip and field border modificaton for management of the wheat stem sawfly (Hymenoptera: Cephidae). *Journal of Entomological Science*. 36:34-45.
- Muesebeck, C. F. W. (1953). Three new reared Braconidae. Washington

Entomological Society Proceedings. 55: 149-151.

Munro, A. J. (1947). Wheat sawfly is on the wing. Quarterly Report, National Federation of Grain Cooperatives. St. Paul, Minnesota. 5(3).

Munro, A. J., W. Nostdahl, and R. L. Post (1949). Wheat stem sawfly. Bimonthly Bulletin. 11(3): 85-91.

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

Nansen, C., M. E. Payton, J. B. Runyon, D. K. Weaver, W. L. Morrill, and S. E. Sing, (2005). Preharvest sampling plan for larvae of the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae), in winter wheat fields. Canadian Entomologist. 137: 602-614.

Neilson, C. L. (1949). Biology and seasonal history of *Pleurotropis utahensis* Crawford, a parasite of the wheat stem sawfly. Canadian Entomologist. 81: 174- 180

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* Norton (Hymenoptera: Cephidae), in western Canada. The Canadian Entomologist 85: 101-107

Nelson, W. A. (1953). Observations on hyperparasitism of the wheat stem sawfly *Cephus cinctus* Norton (Hymenoptera: Cephidae). The Canadian Entomologist. 85: 249-251

Nelson., J. A., K. D. Kephart, A. Bauer, and J. F. Connor (1988). Growth staging of wheat, barley and wild oat: a strategic step to timing of field operations. University of Idaho.

Pesho, G. R., J. U. McGuire, and J. M. McWilliams (1971). Sampling methods for surveys of damage caused by the wheat stem sawfly. FAO Plant Protection Bulletin. 19: 122-130.

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

Riley, C. V., and C. L. Marlatt (1891). Wheat and grass-sawflies. United States Department of Agriculture. Insect Life. 4: 168-179.

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

Runyon, J. B., W. L. Morrill , D. K. Weaver, and P. R. Miller, (2002). Parasitism of

- the wheat stem sawfly (Hymenoptera: Cephidae) by *Bracon cephi* and *B. lissogaster* (Hymenoptera: Braconidae) in wheat fields bordering tilled and untilled fallow in Montana. *Journal of Economic Entomology*. 95: 1130-1134.
- Salt, R. W. (1946). Moisture relationships of the wheat stem sawfly (*Cephus cinctus* Norton): some effects of desiccation. *Scientific Agriculture* 26: 622-629.
- Salt, R. W. (1947). Some effects of temperature on the production and elimination of diapause in the wheat stem sawfly, *Cephus cinctus* Norton. *Canadian Journal of Research* 25: 66-86.
- Salt, N. (1961). A comparison of injury and survival of larvae of *Cephus cinctus* Norton after intracellular and extracellular freezing. *Canadian Journal of Zoology*. 39: 349-357
- Schotzko, D. J., and S. S. Quisenberry (1999). Pea leaf weevil (Coleoptera: Curculionidae) spatial distribution in peas. *Environmental Entomology*. 28: 477-484.
- Sing, S. E. (2002). Spatial and biotic interactions of the wheat stem sawfly with wild oat and Montana dryland spring wheat. Ph.D. dissertation. Land Resources and Environmental Sciences, Montana State University, Bozeman, Montana.
- Sisterson, M. S., and A. L. Averill (2002). Cost and benefits of food foraging for a braconid parasitoid. *Journal of Insect Behavior* 15(4): 571-588.
- Smith, R. W. (1961). Notes on parasites of the wheat stem sawfly, *Cephus pygmaeus* (L.) (Hymenoptera: Cephidae) from continental Europe. *Canadian Entomologist*. 94: 714-717.
- Smith, L., and J. W. Press (1992). Functional response of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae): Influence of host numbers versus host density. *Journal of Entomological Science*. 27:375-382.
- Smith, L. (2004). Temperature influences functional response of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) parasitizing maize weevil larvae in shelled corn. *Annals of Entomological Society of America*. 87: 849-855.
- Somsen, H. W., and P. Luginbill Jr. (1956). *Bracon lissogaster* Mues. a parasite of the wheat stem sawfly. Technical Bulletin 1153. Washington, United States Department of Agriculture.
- Stapel, J. O., A. M. Cortesero, Conseuelo M. De Moraes, J. H. Tumlinson, and W. J. Lewis (1997). Extrafloral nectar, honeydew, and sucrose effects on searching behavior and efficiency of *Microplitis croceipes* (Hymenoptera: Braconidae) in cotton. *Environmental Entomology*. 26(3): 617-623.
- Throne, J. E., D. K. Weaver, V. Chew, and J. E. Baker (1995). Probit analysis of correlated data: multiple observations over time at one concentration. *Journal of Economic Entomology*. 88: 1510-1512.

- Throne, J. E., D. K. Weaver, and J. E. Baker (1995). Probit analysis: assessing goodness-of-fit based on backtransformation and residuals. *Journal of Economic Entomology*. 88: 1513-1516.
- Turnbull, A. L., and D. A. Chant (1961). The practice and theory of biological control of insects in Canada. *Canadian Journal of Zoology*. 39: 697-753.
- Villacorta, A., R. A. Bell, and J. A. Callenbach (1971). Influence of high temperature and light on postdiapause development of the wheat stem sawfly. *Journal of Economic Entomology*. 64(3): 749-751.
- Wallace, L. E. (1962). Field-plot tests of chemicals for wheat stem sawfly control. *Journal of Economic Entomology*. 55(6): 909-911.
- Weiss, M. J., and W. L. Morrill (1992). Wheat stem sawfly (Hymenoptera: Cephidae) revisited. *American Entomologist*. 241-245.
- Wharton, R. A. (1993). Bionomics of Braconidae. *Annual Review of Entomology*. 38: 121-143.
- Winder, L., J. N. Perry, and J. M. Holland (1999). The spatial and temporal distribution of the grain sphid *Sitobion avenae* in winter wheat. *Entomologia Experimentalis et Applicata*. 93: 277- 290.