

THE PATHOGENICITY OF *FUSARIUM* SPP. TO WHEAT STEM SAWFLY,  
*Cephus cinctus* NORTON (HYMENOPTERA: CEPHIDAE)

by

Zhitan Sun

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of

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Dr. David K. Weaver

(Chairperson, Graduate Committee)

Dr. Alan T. Dyer

(Co-chairperson, Graduate Committee)

Approved for the Department of Plant Sciences and Plant Pathology

Dr. John E. Sherwood

Approved for the Division of Graduate Education

Dr. Carl A. Fox

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## ABSTRACT

The wheat stem sawfly is the most destructive insect pest in both winter wheat and spring wheat production in the northern Great Plains. The sawfly is univoltine, and spends all immature stages within protective wheat stems, which explains the difficulty in controlling populations. However, the almost continuous inhabitation of stems also makes larvae more vulnerable to invasion by microorganisms colonizing both living stems and postharvest stubble. *Fusarium* spp. were frequently isolated from fungal-colonized larval cadavers, and were found to be the major lethal factors for overwintering larvae in both laboratory emergence experiments and field surveys over three years. The pathogenicity of single isolates of three *Fusarium* spp., including *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum*, was evaluated against overwintering larvae in sawfly-cut stems and against actively-feeding larvae in growing winter wheat plants over two years. The tested *Fusarium* isolates caused twenty to sixty percent mortality in overwintering larvae, and caused forty to eighty percent mortality in actively-feeding larvae. The *Fusarium* isolates also caused decomposition of sawfly-cut stems and disease in wheat plants, which reflected the versatility of *Fusarium* isolates acting as saprophytes, entomopathogens, and phytopathogens. Deoxynivalenol was detected in wheat stem tissues colonized by the *Fusarium* isolates from two years of field experiments, and deoxynivalenol caused toxicity and inhibited the growth of second and third-instar actively-feeding larvae in laboratory bioassays. Wheat grower observations of greater sawfly infestation in dryland wheat fields led to assessment of larval mortality from *Fusarium* infection in both dryland and irrigated wheat fields. This was studied using cage experiments or field surveys at different locations for three years. Parasitoid attack and fungal infection, mainly by *Fusarium* spp., were found to be the major lethal factors for developing and overwintering larvae, respectively. There was no difference in sawfly survival in dryland or irrigated wheat fields. As ubiquitous soil microorganisms and plant pathogens, *Fusarium* spp. impact wheat stem sawfly populations in the field each year.

## CHAPTER ONE

## INTRODUCTION

Wheat Stem Sawfly Damage

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is a widely distributed and destructive insect pest of wheat production in the northern Great Plains, including Montana, North Dakota, South Dakota, Nebraska, Colorado, Wyoming, Alberta, Saskatchewan, and Manitoba (Weiss and Morrill 1992). The wheat stem sawfly was first reported from the hollow stems of feral grass species, including western wheat grass, *Pascopyrum smithii* (Rydb.), smooth brome, *Bromus inermis* Leyss., and Great Basin wild rye, *Elymus cinereus* (Scribn. and Merr.), in Colorado, Nevada, and California in the late 1800s (Norton 1872). In 1895, sawfly larvae were found infesting spring wheat in north Manitoba (Ainslie 1929). The sawfly is assumed to be native to North America, but there is a hypothesis stating that sawfly was recently introduced from northeastern Asia (Ivie 2001). The sawfly was not a significant pest for a number of years, until the production of cereal grains expanded within the existing range. The rapid increase of wheat acreage, wheat monocultures, introduction of summer-fallow practices, conservation tillage, and prolonged periods of dry weather are primary factors contributing to the increase of sawfly populations. Small, but severe sawfly outbreaks were reported in Alberta, Saskatchewan, and Manitoba between 1906 and 1910, and significant outbreaks occurred in Montana in the 1930s and 1950s (Holmes 1978, Morrill

1983). Excessive wet weather could have negative effects on the activity and population growth of sawfly. Feeding by the larvae at stem nodes forms dark areas visible on the stem exterior, which can be used as visual cues for sawfly infestation (Morrill et al. 1992). The economic losses due to infestation are reductions in grain yield and quality as a result of larval cutting of vascular and parenchyma tissues of wheat stems (Morrill and Kushnak 1996). Estimates of yield losses suggest a 5-15% percent decrease in total seed weight caused directly by larval cutting (Munro et al. 1949). The yield reduction in wheat stems with similar diameters was approximately 11-22% due to a reduction in the number and size of the kernels per head (Holmes 1977). However, the most obvious loss is the lodging of the wheat stems when the larva cuts an internal v-shaped notch at the base of the stems before harvest, which significantly reduces harvest efficiency, including the loss of mature wheat plants, greater fuel consumption, and potential damage to the combine when operated to better collect the lodged stems. There are also increases in the costs of herbicide application to destroy volunteer wheat which grows during the fall and the following spring (McNeal et al. 1955, Morrill et al. 1992, Nansen et al. 2005).

The wheat stem sawfly has remained a serious problem in wheat production for more than one hundred years because it is extremely difficult to control and traditional management tactics are ineffective. Adults emerge from the previous years wheat stubble and the emergence period usually extends from May to July. Female adults fly to the nearby wheat plants and lay one or more eggs into the stem lumen. The larvae feed on the plant tissues inside wheat stems and overwinter as diapausing larvae in the crop residue. These biological characteristics of sawfly could explain the difficulty in controlling

populations. The wheat stem sawfly has caused devastating damage in spring and winter wheat production in Montana and North Dakota in the United States, and Manitoba, Saskatchewan, and Alberta in Canada. Montana suffers the greatest losses with estimated annual loss exceeding 25 million dollars (63 million at 2008 prices), and sawfly infestation levels approaching 100% have been recorded in some wheat fields (Morrill 1994). The greatest yield losses, up to 80%, have also been reported from Montana. Annual losses in all sawfly-infested regions may exceed 100 million dollars (Holmes 1982, Hartel et al. 2003, Weaver et al. 2004). These losses translate to 250 million dollars based on current wheat prices.

#### Wheat Stem Sawfly Description

The wheat stem sawfly is a primitive member of the sawflies belonging to the order Hymenoptera and the family Cephidae. The adults are slender with three yellow bands around a black abdomen. The legs of adults are yellow, and the wings are smoky tinted. Sawfly adults are approximately 10 mm to 15 mm in length. Females tend to be larger than males, and are easily distinguished from males by the presence of an ovipositor at the tip of their abdomen. The wheat stem sawfly was so named because the female cuts into wheat stems to lay eggs with its saw-like ovipositor, which is composed of compressed appendages with serrated teeth (Wallace and McNeal 1966). Unlike most other members of the Hymenoptera, sawflies lack the conspicuous constriction between thorax and abdomen, and the ovipositor does not function as a stinger. The adults are usually observed resting head downward on the stems of grasses, especially cereals. A

mature larva measures 1.5 cm or longer, and is pale white or yellowish in color with a well-defined head.

### Wheat Stem Sawfly Life Cycle

The wheat stem sawfly completes one life cycle per year on its host plants, mainly wild grasses and wheat plants (Ainslie 1920). The life cycle of sawfly includes four stages: egg, larva, pupa, and adult. The wheat stem sawfly completes all immature stages in plant stems, but not the adult stage, and its life cycle is synchronized with the physiological development of host plants. The sawfly adults emerge from obligate diapause in late May to mid-July in areas of Montana, North Dakota, and Canada (Criddle 1915, Wallace and McNeal 1966, Weiss et al. 1990). Male sawflies generally emerge first and remain near field margins where most mating occurs (Holmes et al. 1963, Holmes 1982). The adults are weak fliers, and typically the heaviest sawfly infestations of wheat plants are concentrated at field margins (Holmes 1982). Sawfly infestation in the field has been reported as displaying 'edge effects', where the wheat stems at the field edges have greater infestation, and infestation decreased gradually towards the center of the wheat field (Pesho et al. 1971, Runyon 2001, Sing 2002, Nansen et al. 2005).

The wheat stem sawfly is haplodiploid, male sawflies have only one set of chromosomes (9 chromosomes), while female sawflies have two sets of chromosomes (18 chromosomes). Mating is not required for production of viable eggs because females can control the sex of their offspring by laying either a fertilized (diploid: female) egg or an

unfertilized (haploid: male) egg (Morrill et al. 2000). A typical sawfly population consists of approximately equal numbers of males and females. The earlier emergence of the males ensures that the later emerging females will be mated. However, there is a decrease in the number of the males as emergence progresses, which might result in male-biased populations if the later emerging females are unable to mate (McGinnis 1950, Jacobson and Farstad 1952). A higher ratio of females to males is obtained from the earlier season eggs than that from later season ones (Holmes 1954). Females usually mate once, while successful males may mate with numerous females. Both sexes produce pheromones that are used in mate detection (Bartelt et al. 2002).

The average lifespan of adults is 5-8 days depending on the climatic conditions and available moisture (Criddle 1923). Female adults oviposit heavily during the first 4 days of the flight period, and prefer the longest stems early in the oviposition period, and select less mature stems for oviposition later (Holmes 1978 and 1982, Morrill et al. 1992). Oviposition is reported to occur in the daytime when the air temperature is between 20 °C and 31 °C, and the wind velocity near the ground is below 8 km hr<sup>-1</sup>. Temperature, wind, and precipitation during adult flight can influence sawfly infestation. Low temperatures and heavy wind can delay sawfly infestation, but do not reduce the overall levels of infestation. The amount of precipitation can determine the hardness and thickness of wheat stems, which also affects oviposition and larval fitness (Farstad and Jacobson 1945, Holmes and Peterson 1962, Holmes 1982). Plant developmental stages susceptible to sawfly oviposition begin with stem elongation (Zadoks 31) through anthesis (Zadoks 69) (Zadoks et al. 1974). The females tend to oviposit in the uppermost

internode of each suitable stem. Acceptable stem diameters range from 2.8 mm to 3.4 mm (Holmes and Peterson 1960). The females preferentially choose to lay fertilized eggs in larger stems and unfertilized eggs in smaller stems, which ensures larger female offspring. The sex ratios are male-biased in small wheat stems and female-biased in larger wheat stems. The size, fecundity, and longevity of the female adults increase with the width of the wheat stems, while there is no such relationship between the quality of wheat stems and fitness of the male adults (Morrill et al. 2000, Morrill and Weaver 2000). A healthy female may lay up to 50 eggs, but usually deposits only one egg per stem. Other females may lay eggs in the same stem, but only one larva will survive due to cannibalism of the other immatures present in the same wheat stem (Seamans et al. 1944, Wallace and McNeal 1966).

Newly laid eggs are approximately 1-1.25 mm long and 0.33-0.42 mm wide, milky white, and crescent-shaped (Ainslie 1920). The eggs take approximately 5-8 days to hatch in wheat stems. Newly-hatched larvae are transparent with a light brown head capsule and dark brown eyespots and mandibles. The average size of 1<sup>st</sup>-instar larvae is 2.24 mm in length, and 0.54 mm in width. At maturity, the larvae can reach a length of 13 mm and a width of 2 mm. The sawfly larvae develop within the wheat stems and feed on parenchyma and vascular tissues for a month or longer. Sawfly development includes four or five larval instars, which depends on host plant variety and environmental conditions (Farstad 1940). As the wheat plants start to senesce, the surviving larvae move toward the bottom of the stems in response to the infrared and visible light filtered through the ripening stems decreasing in moisture content (Holmes 1975). After the

larvae approach the soil surface, they turn upwards. As the moisture content of stems decreases to less than 50%, the sawfly larvae will cut a v-shaped notch in the host stems and plug them with frass and plant materials. The girdling sites are generally less than 25 mm above ground level, and the larvae will produce a long, thin, and transparent tube, called a 'hibernaculum', below the girdling site in order to survive the extreme winter weather conditions. The remaining portion of the cut-stem in the ground is called a 'stub', which serves as an overwintering chamber for the sawfly larva. The larvae overwinter in diapause, withstanding temperatures as low as  $-20^{\circ}\text{C}$ . However, low temperature and excessive moisture can also result in high larval mortality (Criddle 1917). Sawfly larval mortality approaches 50% when exposed to temperature of  $-22^{\circ}\text{C}$  for 3 hours. However, most larvae are able to successfully overwinter because they are below the soil surface in the wheat stubble, where temperatures are more moderate than in the air (Morrill et al. 1993).

Pupation occurs in the following spring after spending a minimum of 90 days in diapause at  $10^{\circ}\text{C}$  with 12-15% moisture (Salt 1946 and 1947, Holmes 1978). However, high spring temperatures may trigger the larvae to reestablish diapause and the generation may take two years to complete. In spring, the sawfly larva breaks diapause after exposure to warming weather conditions and increasing day length (Ainslie 1920). In May, the larva begins to enter the pupal stage, and the pupal development lasts from 7 to 16 days depending on the weather, soil moisture, temperature, and other environmental factors (Church 1955, Criddle 1922 and 1923, Holmes 1978). The sawfly pupae are pale or milky white in color with an average length of 12 mm and a diameter of 1.5 mm. After

completing development and eclosion, sawfly adults emerge from May to early July by chewing through and breaching the plugged stubs (Holmes 1954 and 1982).

### Wheat Stem Sawfly Control Methods

With the introduction and establishment of conventional wheat crop-fallow systems in the northern Great Plains, the wheat stem sawfly population was amplified by increased suitable overwintering sites in wheat residue (Holmes and Farstad 1956). Weather conditions, especially rainfall and drought, play important roles in determining the severity of sawfly infestation in wheat. When the weather is dry in the fall or spring, the wheat stems suitable for sawfly attack are few, and the shortage of suitable oviposition sites will result in increased mortality due to cannibalism. Drought in spring will reestablish larval diapause, and may kill the larvae inside the stubs (Seamans 1945). Rainy weather will increase sawfly population size and dispersal because of greater availability of suitable host stems. However, epidemics of wheat pathogens driven by high rainfall will also decrease sawfly infestation in the field (Morrill et al. 1998).

Sampling and monitoring systems should be developed to determine the percentage of plants cut by sawfly per square meter prior to harvest. Although there is no established economic threshold for sawfly, control measures are recommended if 10-15% wheat stems are cut by sawfly, which may produce sufficient sawfly adults to increase the cutting level to 70 percent or more in the following year. There are no effective insecticides for sawfly management, and the acceptable level of sawfly control requires

development and implementation of an integrated approach employing cultural control, resistant varieties, and biological control tactics (Morrill 1995).

### Resistant Cultivars

Currently, one of the most effective ways to reduce damage from the wheat stem sawfly is through the incorporation of sawfly-resistant or solid-stemmed wheat cultivars. Barley, oats, and broadleaf crops, such as canola, flax, and alfalfa, are either less susceptible or not susceptible to sawfly infestation. In 1932, stem solidness was found to be related to sawfly resistance (Kemp 1934). Proliferated pith in the lower three internodes of the stems accounts for resistance to sawfly caused lodging in wheat. The pith accumulated inside the stem makes it difficult for the sawfly larvae to chew through the pith and nodal plates (Farstard 1940, Holmes and Peterson 1962). In addition, excessive moisture in the pith of green plants may kill the sawfly eggs, while the dry pith in mature plants may result in desiccation of descending larvae (Holmes and Peterson 1961). Several resistant cultivars were developed and released after discovery of this trait (Roberts 1954). The first solid-stemmed spring wheat variety, 'Rescue', was developed for resistance to sawfly infestation in 1948, and could decrease infestation by 59% (Platt et al. 1948). Microsatellite markers associated with stem solidness were identified and can be used for selecting solid-stemmed wheat cultivars to manage sawfly (Cook et al. 2004). Female sawflies readily oviposit in solid-stemmed wheat plants, but the newly-hatched larvae are completely surrounded by pith, and tunneling is severely restricted in the pith-filled stems. In addition, the desiccation of the pith during plant maturation may result in larval death because the sawfly larvae can not migrate to the bottom of the solid

stems. Variety trials in the field showed greater mortality of overwintering sawfly larvae in solid stems, while sawfly infestation in solid-stemmed wheat varieties ranged between 16% and 47%, and stem solidness did not affect the rates of parasitism in the field (Morrill et al. 1992 and 1994). There have been many sawfly resistant hard red spring wheat varieties developed. The demand for solid-stemmed winter and spring wheat cultivars has been steadily increasing over the past few years.

In the presence of sawfly infestation, grain producers usually balance potential loss from sawfly feeding versus the loss from growing a lower yielding, resistant cultivar. Sawfly resistant varieties tend to be lower yielding, have reduced disease resistance, and lower protein content. In addition, cloudy weather during stem elongation will result in depressed pith formation in solid-stemmed wheat varieties and reduce resistance to sawfly infestation (Weiss and Morrill 1992). Stem strength and stand density may also be factors in reducing losses caused by sawfly infestation. In a dense crop, uninfested stems can support sawfly-cut stems more effectively than in a thin-stand crop. Another management tactic being explored is using resistant varieties alone or mixed. If the sawfly infestation is slight to moderate, and confined to the field edges, resistant varieties could be planted around the perimeter of the field as trap crops (Morrill et al. 2001), while the whole field should be planted with resistant wheat varieties if the sawfly populations are large (Weiss et al. 1990).

### Cultural Control

Trap Crops Susceptible wheat varieties may be used as trap strips that are grown around the borders of the wheat fields being protected. The trap crops are mowed before the larvae move to the base of the wheat stems (Criddle 1917, Callenbach and Hansmeier 1945). A trap crop, such as smooth brome grass around a field, will attract a sizeable portion of the sawfly population. Trap strips using winter wheat were shown to be effective in protecting spring wheat, and solid-stemmed wheat varieties were effective in reducing sawfly damage by planting them as borders around the hollow-stemmed wheat varieties (Morrill et al. 2001). Trap crops are sometimes recommended for planting 10 to 14 days earlier than the main crop in the field. Planting resistant varieties or non-host plants, such as oats, brome grass, flax, or broad-leaved crop in the trap strips, may also be used because females do not fly far from the sites of emergence (Criddle 1917). However, trap crops are still not favored by grain producers who tend to be unwilling to destroy grain, and trap strips also become ineffective under drought conditions or under pressure from large, dense sawfly populations (Farstad and Jacobson 1945).

Crop Rotation Sawfly damage has become more severe in the conventional wheat crop-fallow system, where suitable overwintering sites are available (Holmes and Farstad 1956). Sawfly has been reported to be able to complete their life cycle on barley also. Adults are reported to be weak fliers that emerge from overwintering residue to infest wheat in nearby fields. Broadleaf crops, including mustard, canola, peas, lentils, and chickpeas are immune to sawfly infestation (Weiss and Morrill 1992). Winter wheat

originally escaped attack by sawflies, but winter wheat in Montana has been consistently attacked by sawflies since 1985 (Morrill and Kushnak 1996). Crop rotation can have negative effects on sawfly populations, but the production of wheat, with limited rotations, is still the most common agricultural practice in the dryland areas of Montana, Alberta, and Saskatchewan.

Delayed Seeding Delayed seeding of spring wheat after May 20<sup>th</sup> will reduce sawfly damage by disrupting the synchronization of sawfly oviposition and wheat development (Weiss et al. 1987). Seeding winter wheat early in the fall or delayed seeding of spring wheat can reduce sawfly infestation to lower levels, and sometimes avoid sawfly attack completely (Morrill and Kushnak 1999). Sawfly infestation levels can also be influenced by seeding rate and row spacing because the females prefer to lay eggs in larger stems, which occurs more frequently in wheat fields with lower seeding rates and wider row spacing (Luginbill 1958). Delayed seeding of spring wheat allows production of two full generations of parasitoids, which accounts for fewer sawflies in the following year. However, a reduced yield can be expected due to a shortened growing season and lack of moisture in July in these semiarid regions (McNeal et al. 1955).

Tillage Shallow tillage in the later summer or early fall will increase larval mortality by exposing stubble on the soil surface and exposing infested stubs to the extreme environmental conditions (Holmes and Farstad 1956, Salt 1961). Sawfly adults are capable of emerging from beneath several inches of soil, which makes cultivating implements less effective (Ainslie 1929). Although deep tillage buries infested stubs and

reduces adult emergence, it may not be a practical method because potential problems with soil erosion. Research has demonstrated that shallow fall tillage provided up to 90 percent sawfly control because of exposure of stubs to hot and dry weather conditions (Morrill et al. 1993). There is about 25 percent sawfly larval mortality if only spring shallow tillage is used. The correct timing of shallow tillage is crucial to increase sawfly larval mortality by achieving the greatest desiccation, and tillage before the larvae enter the prepupal stage will cause a return to diapause (Church 1955, Holmes and Farstad 1956, Holmes 1982). However, conservation tillage and sawfly management by cultivation are incompatible, and shallow tillage also increases labor, machinery costs, and potential soil erosion. Current tillage equipment does not bring enough stubble to the soil surface to reduce sawfly populations in the following year (Goosey 1999).

Swathing Swathing sawfly infested wheat before kernel moisture drops below 40 percent can conserve stems before they are cut by larvae and reduce the overwintering sawfly population (Goosey 1999). Swathing should occur before sawfly lodging occurs and preferably before the larvae have moved below the cutting height (Holmes and Peterson 1965). Swathing has been used by many grain producers to prevent sawfly infested wheat plants from lodging, but different wheat varieties will show varying yield losses when harvested early.

Burning Burning stubble in the autumn or spring was not found to be effective in controlling sawfly because the majority of the stubs are located at ground level. Therefore, burning stubble does not produce enough heat to kill the sawfly larvae inside

the stubs (Ainslie 1920, Criddle 1922). Overall, the negative effects of burning far outweigh the benefits because burning stubble may reduce sawfly populations, but it also destroys overwintering parasitoid populations and reduces the benefit of returning nutrients from the crop residue to the soil.

Sheep Grazing Traditional methods of managing stubble by tilling and burning have negative environmental and economic consequences. Introducing managed grazing on crop residue by sheep after harvest can reduce feeding costs for sheep producers and trim expenditures of wheat grower to manage weed and stubble, and may also disrupt the sawfly life cycle as well. Grazing sheep reduced overwintering sawfly larvae by 68% compared with tillage (47%) (Hatfield et al. 2007). There are many sawfly-infested areas in Montana's billion-dollar grain industry, and sheep grazing could improve the economic and environmental sustainability of Montana's grain enterprises.

### Chemical Control

Insecticides have proven to be an ineffective management tool against sawfly because the adults emerge and lay eggs over a 4-week period and the larvae complete all of their development inside wheat stems, which protects the actively feeding stages from contact or stomach insecticides. Foliar and systemic insecticides, applied as dusts, sprays, or by granular application in soil, have been studied as possible control measures since the 1940s (Skoog and Wallace 1964). High rates of heptachlor were ineffective in controlling sawfly because the mature larvae in higher internodes could survive. Systemic seed treatments were not persistent enough to control oviposition or larval development,

and resulted in significantly decreased plant stands (Wallace 1962). Foliar insecticides, including chlorpyrifos, lambda-cyhalothrin, and carbofuran, also had no significant impact on sawfly population (Holmes and Peterson 1963, Skoog and Wallace 1964, Blodgett 1996). A novel endophytic fungus, *Muscodor vitigenus*, was found to produce known amounts of naphthalene, effectively repelling the adult stage of the sawflies (Daisy et al. 2002). Insecticides with new modes of action are continually tested as they become available. Sawfly adults are rather easy to kill, but efficacy requires application one or two times each week over the whole span of adult emergence to achieve any degree of control, which makes control using insecticides difficult and uneconomical. Therefore, there are no recommended insecticides for application to control wheat stem sawfly (Weiss and Morrill 1992, Morrill 1995).

### Biological Control

Parasitoids and Predators There are naturally occurring enemies of the wheat stem sawfly. Parasitic insects are *Bracon cephi* (Gahan) and *Bracon lissogaster* Muesebeck (Hymenoptera: Braconidae), *Eupelmella vesicularis* Retzius (Hymenoptera: Eupelmidae), *Eupelmus allynii* French (Hymenoptera: Eucharitidae), *Pediobius uthaensis* Crawford (Hymenoptera: Chalcididae), *Pediobius nigratarsis* Thomson (Hymenoptera: Eulophidae), *Eurytoma atripes* Gahan and *Eurytoma parva* Phillips (Hymenoptera: Eurytomidae), and *Scambus detritus* (Holmgren) (Hymenoptera: Ichneumonidae) (Davis et al. 1955, Holmes et al. 1963, Wallace and McNeal 1966, Morrill 1997 and 1998, Runyon et al. 2002). These parasitoids are important factors in suppressing sawfly populations in the native and feral grasses (Criddle 1923 and 1924, Neilson 1949). One predaceous insect is a

clerid beetle, *Phyllobaenus dubius* (Wolcott) (Coleoptera: Cleridae), and its larvae and adults are predatory on sawfly larvae inside the stems. The impact of this predator on sawfly populations needs further research (Morrill et al. 2003).

Parasitic insects are an important factor in sawfly populations in feral grasses where parasitism levels sometimes approach 100% (Morrill 1997). Until recently, the number of parasitized sawflies gradually increased as the parasitoid populations followed the sawflies into wheat production (Neilson 1949, Holmes 1953, Somsen and Luginbill 1956). Reductions in sawfly infestations in wheat have been attributed to heavy parasitism in the same or in the immediately preceding year. Different parasitoid species vary in their effects on sawfly populations. Two species of parasitic wasps, *Bracon cephi* and *Bracon lissogaster*, are commonly found parasitizing sawfly larvae in wheat, and both of these larval parasitoids are protelean, host-specific idiobiont ectoparasitoids that have been reported to efficiently suppress sawfly populations in Canada and Montana (Ainslie 1929, Nelson and Farstad 1953, Somsen and Luginbill 1956, Holmes et al. 1963, Morrill et al. 1994). *B. cephi* was described first as *Microbracon cephi* from sawfly infested grasses, and both the males and females of *B. cephi* usually have a yellowish orange body (Gahan 1918). The description of *B. lissogaster* was based on a study of 17 females and 28 males that were reared from sawfly infested grasses collected near Choteau, Montana. The male of *B. lissogaster* typically has a dark head and thorax with a yellowish orange metasoma, and the female has a black head with an orange body (Muesebeck 1953). The biology of *B. cephi* and *B. lissogaster* are similar, except that females of the former lay one egg per paralyzed sawfly larva, while females of the latter

lay one to four eggs per host larva. Therefore, *B. lissogaster* is a frequently gregarious ectoparasitoid and sometimes three to four adult parasitoids can be developed on a single sawfly host. Because of this gregarious parasitism, the size of the larvae and adults is typically smaller than those feeding in isolation. Cannibalism is not evident among the parasitoid larva. Both *B. cephi* and *B. lissogaster* are native wasps that attack the developing sawfly larvae inside the wheat or grass stems and the level of parasitism ranged from 15% to 98% in wheat fields in Montana (Morrill et al. 1994, Morrill et al. 1998). The benefits of parasitism can not be realized until the parasitoid populations increase to effective levels in the years following heavy infestation.

*B. cephi* and *B. lissogaster* both have two generations annually. The first generation emerges from overwintering stubble in June and July when sawfly adults are present. The female parasitoid adult has been observed tapping the wheat stems with its antennae to locate the sawfly larvae, and ovipositing one or more eggs on or near the paralyzed sawfly larva. The distributions of the parasitoid larvae in stems are influenced by the feeding locations of the sawfly larvae at the time of oviposition by the female parasitoids, and first generation parasitism tends to occur in the upper internodes of the host plants, while the second generation tends to be in the lower internodes of host plants (Holmes et al. 1963). A newly hatched parasitoid larva will feed externally on the paralyzed sawfly larva with its mandibles for 6-10 days, and only the integument of the host remains after feeding. The mature larva will migrate up the stem where it spins a cocoon, which is followed by adult emergence after metamorphosis (Nelson and Farstad 1953, Somsen and Luginbill 1956, Morrill et al. 1998). The parasitoid adults emerge by

chewing a circular hole through the cocoon and stem. The male adults can live for 2 weeks, and the female adults can live approximately 4 weeks under laboratory conditions. The parasitoid adults feed on water droplets and nectar, and fly for considerable distances. The rate of parasitism increases after rainfall, which might be due to increased adult activity and better oviposition in softened wheat stems (Nelson and Farstad 1953). If there is an extended growing season allowing for longer availability of green wheat and grass stems, the second generation of parasitoids appears in mid-August when the wheat plants are maturing and the sawfly larvae are moving down the wheat stems, so the sawfly larvae tend to be more protected as they descend further. Therefore, if the wheat ripens early, there will be a corresponding reduction in parasitoid populations in the subsequent year. However, the second generation of parasitoids can usually survive in nearby grasses because these retain higher moisture levels until later into the summer (Holmes et al. 1963). Increasing sawfly populations in the field can cause low levels of parasitism, which might result from the cannibalism of paralyzed, parasitized larvae by actively feeding larvae and by inefficiency of locating hosts by parasitoid adults due to the increased concentrations of chemical cues in the field, as has been reported for other systems (Flinn and Hagstrum 2002, Smith 2004). Hyperparasitoids on sawfly parasitoids, including *Eupelmella vesicularis* Retzius and *Eurytoma attripes* Gahan, have been reported to influence the effectiveness of biological control of wheat stem sawfly (Nelson 1953). In addition, the parasitoids overwinter in standing stems rather than the stubble, so one way to increase parasitoids population is to leave a greater portion of wheat stems intact during harvest (Morrill 1997, Morrill et al. 1998).

Pheromone Traps Understanding the chemical ecology of the wheat stem sawfly could lead to new and environmental-friendly approaches for reducing agricultural losses. Both sawfly sexes produce pheromones composed of similar constituents, and these chemical signals trigger behaviors and interactions in both sexes (Borden et al. 1978). Pheromones are released from the waxy layer of the sawfly exoskeleton. Groups of males can form localized leks in their fight behavior as a result of the pheromones from other males, which can increase the concentration of male pheromones, and make female sawflies posture and release pheromones. This allows the males to approach females and mate with females on the wheat residue or growing wheat stems.

The cuticular lipids of the sawfly were investigated by coupled gas chromatography–mass spectrometry (GC-MS), and found to be composed mainly by *n*-alkenes and *n*-alkanes (Bartelt et al. 2002). The major compounds of cuticular extracts showed no GC-electroantennographic detector (GC-EAD) activity, but ozonolysis of extracts gave dramatically increased amounts of GC-EAD-active materials. Volatile collections from male and female sawflies contained the same GC-EAD-active compounds that stimulated both male and female antennae, these compounds included 9-acetyloxynonanal, 13-acetyloxytridecanal, aldehydes with 9–16 carbon chain lengths, acids with 8–10 carbon chain lengths, and phenylacetic acid. 9-acetyloxynonanal was shown to cause the strongest EAD response, and a field bioassay demonstrated the attractiveness of 9-acetyloxynonanal for both male and female sawflies when synthetic compounds were used in baited traps (Cossé et al. 2002).

Plant Semiochemicals Plant semiochemicals can have a significant effect on the living organisms that feed on plants, these semiochemicals include oviposition deterrents, toxins, defensive proteins, and other compounds (Dicke and van Loon 2000, Turlings and Benrey 1998). Wheat is known to give off volatiles due to herbivory and disease stresses, and these volatiles are part of the defensive responses to the insect injury (Buttery et al. 1985). There are certain known volatiles produced by wheat and oats, including aliphatic aldehydes and ketones, aliphatic alcohols and esters, terpenoids and aromatic compounds (Buttery et al. 1982). Some chemical differences are quantitatively apparent in the volatile chemicals produced by various host species and may include semiochemicals used by sawflies and parasitoids. Research on herbivore-induced volatiles could enhance the understanding of sawfly-wheat-parasitoid interactions and lead to more effective control measures for the sawflies. The use of pheromone and plant volatiles in insect trapping has been reported to be effective when applied at small scales (Bartelt et al. 2002). These traps can be used to monitor and reduce populations in the field. Herbivore-induced plant volatiles also play important roles in attracting parasitoids and predatory insects to detect host pests (Mayland et al. 2000). First, parasitoids use vision, mechano-reception or chemo-reception to pinpoint the location of their hosts. Once the habitat containing hosts has been located, parasitoids have been shown to be very efficient in using volatile chemicals emitted by herbivore-damaged plants to locate individual hosts (Turlings and Benrey 1998). Parasitoids and predators of various life stages can also be attracted by using synthetic herbivore-induced plant volatiles (James 2003a and 2003b). Therefore, it may be possible to attract parasitoids to sawfly infested fields using

synthetic herbivore-induced plant volatiles. Repellency of some pests due to induced plant volatiles has been well studied. For example, the induced plant volatile cis-jasmone repelled aphids from plants in both laboratory and field trials (Birkett et al. 2000, Bruce et al. 2003). The oviposition behavior of herbivores can also be influenced by the chemical cues of plants in response to infestation. Adults of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) can differentiate the host plants with or without larvae when seeking for oviposition sites (Anderson and Alborn 1999, Jonsson and Anderson 1999). If female sawflies are able to distinguish the volatiles between wheat stems containing sawfly larvae and stems without sawfly larvae, the repellents could be used to trigger the rejection of wheat stems.

*Fusarium* Species The life cycle of wheat stem sawfly may help to explain the difficulty in controlling populations because all immature stages occur within the protective wheat stem. The almost continuous inhabitation of stems also makes larvae more vulnerable to invasion by microorganisms colonizing both living stems and postharvest stubble. According to previous field observations, there were dead and colonized sawfly larvae inside of the wheat stubble in the post-harvest wheat fields. *Fusarium* isolates, belonging to the species of *F. graminearum* Schwabe Gr1 (syn. *pseudograminearum*), *F. culmorum* (W. G. Smith) Sacc., *F. acuminatum* Ell. & Ev. sensu Gordon, *F. avenaceum* (Fr.) Sacc., and *F. equiseti* (Corda) Sacc. sensu Gordon, were isolated from those colonized larval cadavers (Wenda-Piesik et al. 2006). These *Fusarium* spp. are generally known to cause *Fusarium* crown rot and *Fusarium* head blight of wheat in Montana, but they are also vigorous saprophytic colonizers of wheat

straw in the field (Walker 1941, Hogg et al. 2007). The straw penetration rate of *Fusarium* spp. is closely correlated with cellulolysis, suggesting the speed of penetration depends on rate of enzymatic degradation of the cell walls around the apices of penetrating hyphae. In nitrogen deficient situations, *Fusarium* spp. are able to produce chitinases to recycle nitrogen from chitin and from other nitrogen bearing compounds in wheat straw (Garret 1975a and 1975b). The ability to colonize wheat stubble distinguishes *Fusarium* spp. from other potential entomogenous microorganisms attacking sawfly larvae. The saprophytic characteristics enable *Fusarium* spp. to invade wheat stubble, and subsequently, to attack sawfly larvae inside stubble. The pathogenicity of these *Fusarium* isolates to the sawfly larvae was confirmed by laboratory bioassays, and more than 80% larval mortality was observed after 13 days in *Fusarium* treatments (Wenda-Piesik et al. 2006).

*Fusarium* is a large genus of filamentous fungi widely distributed in soil worldwide, but most species are harmless saprobes and are relatively abundant members of soil microbial communities. *Fusarium* spp. produce macroconidia and chlamydospores that help them to survive and remain biologically active over an unusually wide range of temperature and moisture levels for years (Wilcock and Megan 2001). As plant pathology and entomology have been traditionally pursued as independent disciplines, the dual role of acting as both plant pathogens and insect pathogens by certain *Fusarium* spp. has not been widely studied. *Fusarium* spp. as saprobes and plant pathogens, were frequently isolated from stems, stubble, and crowns of wheat plants, including *F. pseudograminearum*, *F. culmorum*, *F. acuminatum*, *F. avenaceum*, and *F. equiseti*

(Klaasen et al. 1991, Luque et al. 2005). Among these saprobes and plant pathogens, some *Fusarium* spp. selectively parasitize specific plants, animals, or insects if these suitable hosts are encountered. For example, *F. oxysporum*, a soil saprobe, has received considerable attention from both plant pathologists and entomologists over the past half century because of the ability to cause vascular wilt and root rot diseases in plants, and also mycosis of insects. Some *F. oxysporum* strains have been used as herbicidal agents because they selectively kill the weeds *Orobanche cumana* Wallr. and *Striga hermonthica* (Del.) Benth. in the dry zones of Africa, but some *F. oxysporum* strains were studied in France, India, and Russia for control of mosquito larvae as they were highly virulent to some mosquito species (Hasan and Vago 1972, Kistler 1997, Thomas et al. 1998, Elzein and Kroschel 2006, Elzein et al. 2006). Other common *Fusarium* saprobes, like *F. pseudograminearum*, *F. culmorum*, *F. acuminatum*, *F. avenaceum*, and *F. equiseti*, are regarded as plant pathogens since they cause *Fusarium* crown rot and *Fusarium* head blight of wheat and also other plant diseases worldwide, but they also occasionally cause epizootics of insect disease in the field (Strongman et al. 1988, Dowd et al. 1989). Certain *Fusarium* isolates exist symptomlessly within crop plants as endophytes, and possibly even enhance the growth of infected plants (Bacon and Hinton 1996, Yate et al. 1997). Endophytic *F. oxysporum* strains have been studied in Africa to control the burrowing nematode *Radopholus similis* in banana plantations (Athman et al. 2006). Some insects also contribute to the dispersal of *Fusarium* spp. in the environment by spore passage through their guts (Teetor-Barsch and Roberts 1983). Entomogenous fungi could have evolved from phytopathogenic fungi via adaptations in extracellular

hydrolytic enzymes so as to accommodate hydrolysis of proteinaceous insect cuticles. Many of the plant pathogens possess structural and behavioral adaptations which are very similar to entomopathogens, and the underlying mechanisms of fungal pathogenesis may be similar in insects and plants. Many *Fusarium* spp. are known to produce a broad spectrum of protein and polysaccharide-hydrolysing enzymes, which could be useful in complete hydrolysis of complex organic substances, including both living and non-living plant cell walls and insect cuticles. This versatility of *Fusarium* spp. in transforming from plant pathogens to insect pathogens enables them to cause epizootics of both plant diseases and insect diseases in fields (St Leger et al. 1997).

In addition to the insecticidal activities of extracellular hydrolytic enzymes, six to eight genera of plant pathogenic fungi, including *Fusarium* and *Aspergillus* spp., produce mycotoxins, such as trichothecenes, fumonisins, and beauvericin, which may be active against both plants and insects (Diener et al. 1987, Abbas and Mulrooney 1994, Ganassi et al. 2001). Phytopathogenic *Fusarium* spp. and their corresponding insecticidal mycotoxins are shown in Table 1.1. For example, *Fusarium graminearum* Schwabe is a pathogen of wheat, corn, and other cereals in northern temperate climates and could reduce the quality of grain primarily because this fungus produces mycotoxins, including trichothecene deoxynivalenol, zearalenone, and approximately 40 other secondary metabolites, all of which could be toxic to both plants and insects (Dowd et al. 1989). Fusaric acid is a well-known phytotoxin that is produced by several *Fusarium* spp., causing wilt diseases in a great variety of plants. It has weak insecticidal activity in insects, but it can synergize the toxic effects of plant allelochemicals. A case in point is

that fusaric acid can synergize the toxicity of the co-occurring cotton allelochemical gossypol, and increase the mortality of *Heliothis zea* (Boddie) larvae, which might result from fusaric acid inhibiting the oxidative enzymes responsible for gossypol detoxification in the insect (Dowd 1989). Beauvercin, a cyclic hexadepsipeptide, was isolated from *F. semitectum* Brek. ex. Rav. and from *F. subglutinans* (Wollenw. et Reinking) using a bioassay for toxicity to Colorado potato beetle *Leptinotarsa decemlineata* (Say), and its insecticidal properties have been widely studied using various invertebrate bioassays. However, beauvercin also plays an important role in the etiology of plant disease as beauvercin is toxic to protoplasts and causes cell death in many plants, and is produced by both entomopathogenic and phytopathogenic *Fusarium* spp., including *F. acuminatum*, *F. avenaceum*, and *F. equiseti* (Logrieco et al. 1998, Paciolla et al. 2004).

Table 1.1. Phytopathogenic *Fusarium* spp. and their corresponding insecticidal toxins.

Species	Toxins	Insects	References
<i>F. acuminatum</i>	HT-2 toxin, T-2 toxin	<i>Tenebrio molitor</i>	(Davis and Smith 1977)
<i>F. avenaceum</i>	Enniatin	<i>Choristoneura fumiferana</i>	(Strongman et al. 1988)
<i>F. equiseti</i>	Trichothecene	<i>Hymenoptera, Lepidoptera</i>	(Kalvish 1979)
<i>F. graminearum</i>	Dihydroxycalonectrin vomitoxin, culmorin, zearalenone	<i>Spodoptera frugiperda,</i> <i>Heliothis zea,</i> <i>Tribolium confusum</i>	(Dowd et al. 1989, Harein et al. 1970, Eugenio et al. 1970)
<i>F. lateritium</i>	Trichothecene, enniatin	<i>Oulema gallaeciana,</i> <i>Hemiberlesia rapax,</i> <i>Lucilia sericata</i>	(Miczulski et al. 1977, Burmeister et al. 1987, Cole and Rolinson 1972)
<i>F. moniliforme</i>	Beauvericin	<i>Leptinotarsa decemlineata,</i> <i>Mylabris pustulata,</i> <i>Leucinodes orbonalis,</i> <i>Scirpophaga incertulas</i>	(Beevi and Jacob 1982, Gupta et al. 1991, Yasodha et al. 2004)
<i>F. nivale</i>	Vomitoxin, T-2 toxin	<i>Tenebrio molitor</i>	(Davis et al. 1975)
<i>F. oxysporum</i>	Trichothecene, T-2 toxin, AAL-toxin	<i>Aedes detritus,</i> <i>Heliothis virescens</i>	(Lynch et al. 1978, Abbas et al. 1994)
<i>F. polyphialidicum</i>	Fumonisin	<i>Lymantria dispar</i>	(Hajek et al. 1990)
<i>F. proliferatum</i>	Fumonisin B <sub>1</sub> , beauvericin	<i>Schizaphis graminum</i>	(Ganassi et al. 2001)
<i>F. sambucinum</i>	Trichothecene	<i>Lymantria dispar</i>	(Hajek et al. 1993)
<i>F. semitectum</i>	Beauvericin,	<i>Leptinotarsa decemlineata,</i> <i>Hymenoptera, Lepidoptera</i>	(Grove and Pople 1980, Kalvish 1979)
<i>F. solani</i>	Fusarubin, javanicin, fusaric acid	<i>Scolytus scolytus,</i> <i>Calliphora erythrocephala</i>	(Barson 1976, Claydon et al. 1976)
<i>F. sporotrichioides</i>	Trichothecene, T-2 toxin	<i>Choristoneura fumiferana,</i> <i>Tenebrio molitor</i>	(Strongman et al. 1990, Davis and Smith 1981)
<i>F. verticillioides</i>	Fumonisin	<i>Eldana saccharina</i>	(McFarlane et al. 2005)

### Research Goals

Ubiquitous *Fusarium* spp. in the soil are important factors impacting wheat stem sawfly populations. Previous research in our laboratory reported fungal-colonized larval cadavers infected by *Fusarium* spp. inside sawfly-cut stems collected from postharvest wheat fields. The goals of this study were to investigate the pathogenicity of *Fusarium* spp. to wheat stem sawfly, and the occurrence frequency of *Fusarium* infection among sawfly larvae in wheat fields. Therefore, in chapter two, the occurrence frequency of *Fusarium* infection among overwintering sawfly larvae in the field was investigated by both laboratory emergence experiments and field surveys. In chapter three and chapter four, the pathogenicity of the isolates of *Fusarium* spp., obtained from fungal-colonized larval cadavers of wheat stem sawfly, was tested against the overwintering sawfly larvae in a postharvest environment and also against actively-feeding larvae during the growing season. Deoxynivalenol was detected in the growing wheat stem tissues colonized by the *Fusarium* isolates. Subsequently, in chapter five, the toxicity and growth inhibition effects of deoxynivalenol on developing sawfly larvae were explored in laboratory bioassays. According to wheat grower observations, there is greater sawfly infestation in dryland as opposed to irrigated wheat fields. In chapter six, sawfly mortality due to *Fusarium* infection in both dryland and irrigated wheat fields was studied using both cage experiments and field surveys.

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## CHAPTER TWO

INCIDENCE OF *FUSARIUM* COLONIZATION IN THE OVERWINTERING  
MORTALITY OF LARVAL WHEAT STEM SAWFLIES, *Cephus cinctus* NORTONAbstract

Major lethal factors contributing to overwintering mortality of larval wheat stem sawflies were evaluated using laboratory emergence experiments and field surveys for three years. Previous research had shown that *Fusarium* spp. are pathogenic to diapausing larvae. Recurring colonization in the field indicated that fungal infection was the most important lethal factor in larval populations over the winter, and could account for ten to twenty percent of larval mortality. Other factors impacting overwintering larval mortality were braconid parasitoids, a coleopteran predator, mechanical damage, and environmental factors. The fungi that were isolated from colonized larval cadavers included *F. culmorum*, *F. pseudograminearum*, *F. acuminatum*, *F. avenaceum*, and *F. equiseti*. *Fusarium* spp. were the dominant microorganisms isolated from colonized larval cadavers. Widespread, naturally-occurring *Fusarium* spp. are consistently impacting overwintering annual wheat stem sawfly larval populations in cropland.

Introduction

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is a widely distributed and destructive insect pest of wheat grown in most of the northern

Great Plains, including Montana, North Dakota, South Dakota, Nebraska, Colorado, Wyoming, Alberta, Saskatchewan, and Manitoba (Weiss and Morrill 1992). Direct economic losses due to the sawfly infestation are reductions in kernel weight and grain quality because larval feeding in the parenchymous and vascular tissues of wheat stems disrupts the movement of nutrients (Morrill and Kushnak 1996). An even more apparent loss is the lodging of mature stems that is caused by larval cutting at the base of the stems just before harvest, resulting in unrecoverable, mature grain. Annual losses in all sawfly-infested wheat production regions may exceed 100 million dollars (Hartel et al. 2003, Weaver et al. 2004), which is 250 million dollars at current prices. The wheat stem sawfly completes one life cycle per year in wheat or suitable grass hosts. Sawfly adults emerge from obligate diapause in the wheat residue remaining from the previous year between late May and July, and females tend to deposit an egg in the uppermost internode of each stem (Weiss et al. 1990). At hatch, the larva begins feeding on the parenchyma cells and will continue feeding on both vascular and parenchymous tissues in wheat stem for a month or longer (Farstad 1940). At plant maturity, the larva descends within the stem and cuts an internal v-shaped notch at the stem base just before harvest (Holmes 1975). Mature larvae overwinter in diapause for 8 to 9 months at or the below soil surface in wheat residue. Metamorphosis begins as the temperatures warm in the early spring of the next year (Wallace and McNeal 1966, Holmes 1975). There are naturally occurring enemies of the wheat stem sawfly. Parasitic insects, mainly *Bracon cephi* (Gahan) and *Bracon lissogaster* Muesebeck, attack the developing larvae inside stems and the levels of parasitism ranged from 15% to 98% in fields in Montana (Morrill

et al. 1994, Morrill et al. 1998). A clerid beetle, *Phyllobaenus dubius* (Wolcott), is occasionally predatory on sawfly larvae inside of the sawfly-cut wheat stems, or ‘stubs’ (Morrill et al. 2003).

The biological characteristics of wheat stem sawfly could explain the difficulty in controlling populations because all immature stages occur within the protection of wheat stems. However, long-term overwintering within wheat residue also exposes larvae to invasion by soil microorganisms, which includes saprophytic, entomogenous, and phytopathogenic fungi. Previous observations indicated dead and colonized larvae were present inside stubs in postharvest wheat fields. Several *Fusarium* spp. isolates, including the following species: *F. graminearum* Schwabe Gr1 (syn. *pseudograminearum*), *F. culmorum* (W. G. Smith) Sacc., *F. acuminatum* Ell. & Ev. sensu Gordon, *F. avenaceum* (Fr.) Sacc., and *F. equiseti* (Corda) Sacc. sensu Gordon, were obtained from the larval cadavers. The pathogenicity of these *Fusarium* isolates to diapausing sawfly larvae was confirmed in laboratory bioassays and more than 80% larval mortality was observed after 13 days in the *Fusarium* treatments (Wenda-Piesik et al. 2006; In preparation).

*Fusarium* is a large genus of filamentous fungi that is widely distributed in soil and occurs worldwide (Burgess et al. 1988). Most species are harmless saprophytes and are relatively abundant members of soil microbial communities (Elmholt 1996, Pankhurst et al. 1996). *Fusarium* species are generally known to cause plant disease, such as *Fusarium* head blight, *Fusarium* crown rot, and vascular disease (Nelson et al. 1981, Hogg et al. 2007), but they also occasionally cause epizootics of insect disease in the field (Strongman et al 1988, Dowd et al 1989). There have not been any comprehensive

studies on the colonization of overwintering wheat stem sawfly larvae by *Fusarium* species. In these studies, we report on the lethal factors contributing to overwintering larval mortality, including the proportion of larval cadavers that were colonized by naturally-occurring, entomopathogenic *Fusarium* species. Laboratory emergence experiments and field surveys were conducted from 2005 to 2007.

## Materials and Methods

### Sawfly Larvae in Diapause

Stubs containing larvae were collected from postharvest wheat fields at locations in Montana in March of 2005, 2006, and 2007 (Figure 2.1). Stubs were identified and sorted from other wheat residue using the presence of characteristic precise uniform cuts and by characteristic frass plugs at the apices of the stubs. The stubs were used in adult emergence experiments or were dissected to determine mortality levels.

### Emergence Experiments

Emergence experiments were conducted at Montana State University. Intact stubs, showing no obvious mechanical damage and no evidence of fungal attack, were individually placed in glass vials (2 cm wide and 6 cm long) capped with cotton plugs, and held at 23 °C with a photoperiod of 12L:12D. The cotton plugs were moistened with water every 3 days to provide humidity to facilitate adult emergence. Moisture and temperature are two important factors that determine pupation and subsequent adult emergence from the stubs. Sawfly adults normally emerged after 6 weeks at this temperature, with an emergence interval lasting about two weeks. The percentage of

adults emerged was recorded for the total number of intact stubs. Stubs that failed to yield adults were dissected to determine the factors causing larval mortality. Fungal infection, parasitoid, and predator attack are the three major lethal factors observed and these were confirmed by the presence of fungal-colonized larval cadavers, parasitoid cocoons, and predator frass in the stubs, respectively. Other factors, including drowning (cadaver saturated with water) and mechanical damage (desiccated cadavers in broken stubs) also accounted for larval mortality. Larval cadavers with obvious fungal mycelia on the surface were held separately in disposable sterile centrifuge tubes (2ml) for subsequent fungal isolation.

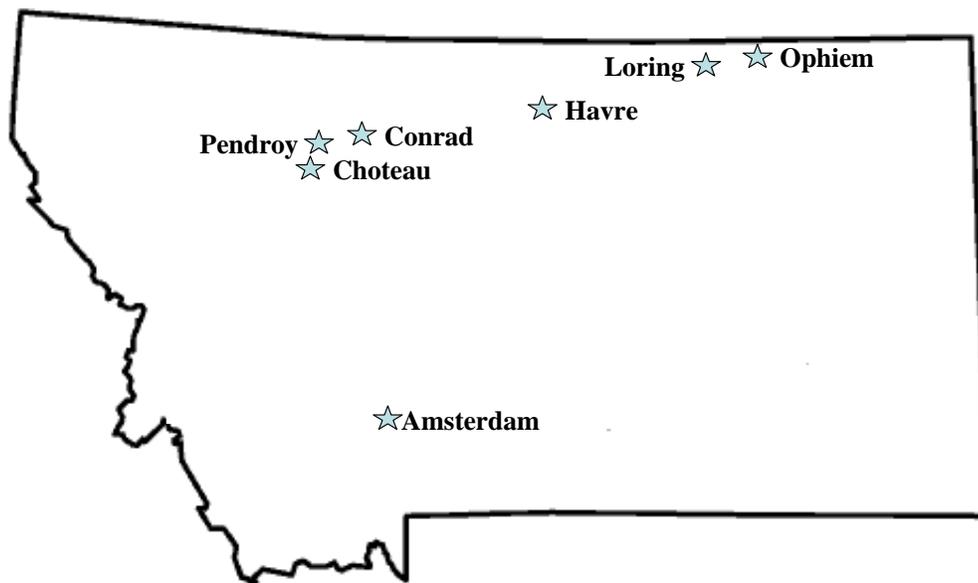


Figure 2.1. Location of field sites in Montana where wheat stem sawfly-cut stems were collected in 2005, 2006, and 2007.

### Field Surveys

These focused on the overwintering mortality caused by fungal attack of the larvae in diapause. Overwintered stubs were collected from 3 or 4 randomly selected locations at each postharvest wheat field in March of 2006 and 2007. The newly unearthed stubs were placed in sample bags and labeled by collection location for each field. Subsequently, thirty to forty stubs from each field location were individually dissected to determine the percentage of larval cadavers colonized by fungal infection in all the larvae found in stubs. Colonized larvae with obvious fungal mycelia were saved separately in disposable sterile centrifuge tubes (2ml) for subsequent fungal isolation.

### Fungal Isolation and Identification

Fungal-colonized larval cadavers were individually surface-sterilized by immersion in 80% ethanol for 5 minutes and then dried under a filtered air flow for 20 minutes. The larval cadavers were then plated on water agar supplemented with 50 µg chloramphenicol per ml. After three days incubation at 23 °C, a mycelial tip from each fungal isolate growing out of the larval cadavers was transferred to a potato dextrose agar (PDA) slant for preliminary identification to genus (Barnett and Hunter 1972). Isolates of *Fusarium* were prepared using single conidia techniques on PDA and carnation leaf agar slants (Nelson et al. 1983). The identification of each *Fusarium* species isolated was confirmed by the *Fusarium* Research Center at Pennsylvania State University.

### Statistical Analysis

Data from the emergence experiments in 2005, 2006, and 2007 were analyzed using a  $\chi^2$  test to determine significant differences in sawfly mortality between the sites ( $P < 0.05$ ). The percentage data for colonized larvae from the field survey work in 2006 and 2007 were transformed with arcsine square-root transformations, and then analyzed using analysis of variance (ANOVA) (PROC MIXED, SAS Institute 2001). Multiple comparisons were made between the sites by separating the means using the Fisher least significance difference (LSD) at  $\alpha = 0.05$ .

### Results

In 2005, the emergence experiment used stubs collected from 5 sites in Montana (Table 2.1). Adult emergence was greater than 90% for most sites, and there was no significant difference between the sites ( $\chi^2 = 0.99$ ,  $df = 4$ ,  $P > 0.05$ ). The percentage of larvae colonized by fungi ranged between 1.8% to 3.6% across sites, and no significant difference was evident between the sites ( $\chi^2 = 0.94$ ,  $df = 4$ ,  $P > 0.05$ ). For parasitoids, Ophiem site had the greatest percentage of parasitism, and was significantly different from other sites ( $\chi^2 = 13.44$ ,  $df = 4$ ,  $P < 0.05$ ). Predators were rarely found in stubs and were not abundant enough for statistical comparisons. There was no significant difference between sites for other mortality factors, including drowning and mechanical damage ( $\chi^2 = 2.64$ ,  $df = 4$ ,  $P > 0.05$ ). The fungi isolated from the colonized larvae and their frequency of occurrence in the 2005 adult emergence experiment is shown in Table 2.2. Members of the genus *Fusarium* were clearly the dominant fungi isolated across the sites,

including isolates of *F. culmorum*, *F. pseudograminearum*, *F. acuminatum*, *F. equiseti*, *F. avenaceum*, and other unidentified *Fusarium* species. Isolates of *Paecilomyces* spp. and *Monilia* spp. were also obtained from fungal-colonized larval cadavers.

Table 2.1. Occurrence frequency (%) of mortality factors in sawfly-cut stems in 2005 and 2006.

Overwinter status	Sites					
	2005					2006
	Amsterdam	Conrad	Havre	Ophiem	Pendroy	Amsterdam
Emerged adults	94.53	92.75	94.09	82.93	90.86	86.22
Colonized larvae	2.01	3.62	1.97	2.44	1.77	8.11
Parasitized larvae	0.55	0.91	1.38	8.13*	4.13	0.22
Predated larvae	0.18	0	0	0.81	1.18	0
Other mortality factors	2.74	2.72	2.56	5.69	2.06	4.61

In 2005 the number of sawfly-cut stems from Amsterdam, Conrad, Havre, Ophiem, and Pendroy was 548, 552, 508, 123, and 339, respectively; in 2006 the number of sawfly-cut stems from Amsterdam was 456.

\*Indicates significant difference between values in the row using a chi-square test ( $P < 0.05$ ).

Table 2.2. The number of fungal isolates from colonized larval cadavers within sawfly-cut stems at locations across Montana in 2005.

Fungal species	Sites					Overall colonization (%)
	Amsterdam	Conrad	Havre	Ophiem	Pendroy	
<i>F. culmorum</i>	1	13	0	0	4	29.51
<i>F. pseudograminearum</i>	0	2	0	0	1	4.92
<i>F. acuminatum</i>	2	1	3	1	0	11.48
<i>F. avenaceum</i>	7	4	4	2	0	27.87
<i>F. equiseti</i>	0	5	0	0	1	9.84
<i>Fusarium</i> spp.	3	2	1	1	0	11.48
<i>Paecilomyces</i> spp.	0	1	0	0	1	3.28
<i>Monilia</i> spp.	0	0	1	0	0	1.64

In 2006, the percentage of emerged adults, colonized larvae, and parasitized larvae was 86.22%, 8.11%, and 0.22% respectively for stubs collected postharvest in a wheat field near Amsterdam, MT (Table 2.1). No predation was found in these stubs, and other factors accounted for 4.6% larval mortality. In the field survey, we noticed

aggregated distributions of moderately decomposed (dark-colored and noticeably degraded) stubs colonized by fungi in the postharvest wheat field. Stubs were collected from field areas with slightly and moderately decomposed wheat residue. The percentage of fungal-colonized larvae from the slightly decomposed areas was significantly lower than the moderately decomposed areas ( $F = 455.81$ ,  $df = 1$ ,  $P < 0.001$ ) (Figure 2.2). Isolates of *Fusarium* spp. were the dominant fungi isolated from colonized larvae in both the emergence experiment and the field survey in 2006 (Table 2.3). Isolates of *Penicillium* spp. were also obtained from colonized larval cadavers.

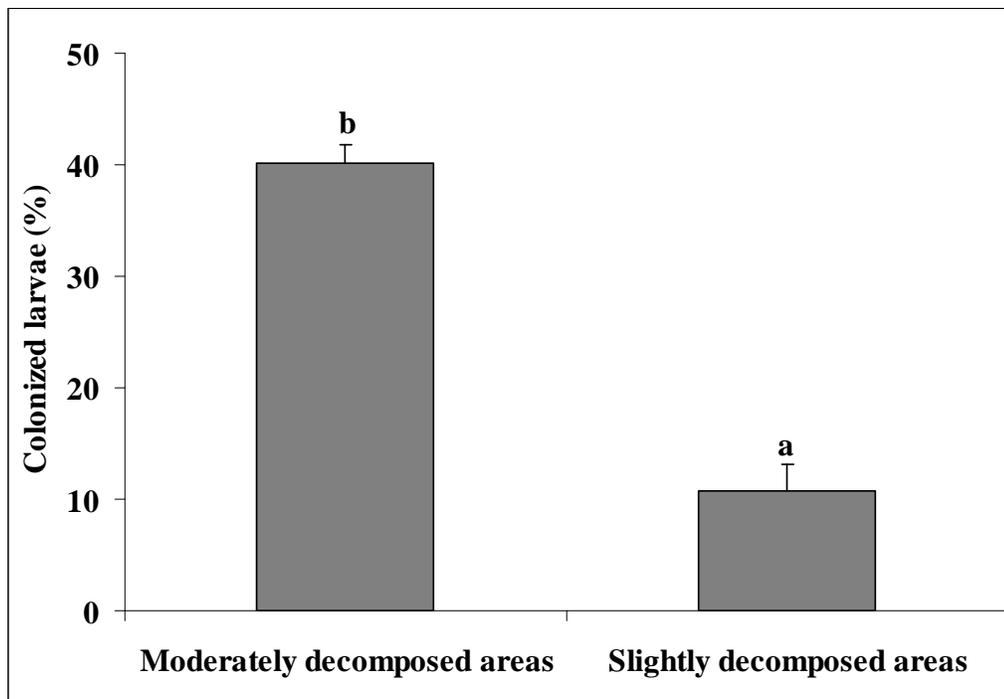


Figure 2.2. The percentage of fungal-colonized larvae within sawfly-cut stems in field areas of slightly and moderately decomposed residue from a 2006 field survey near Amsterdam, MT.

Means for slightly decomposed (b) and moderately decomposed (a) field areas are significantly different ( $LSD = 2.89$ ).

Table 2.3. The number of fungal isolates from colonized larval cadavers within sawfly-cut stems in emergence experiments and field samples from near Amsterdam, MT in 2006.

Fungal species	Emergence experiment	Field survey	Overall colonization (%)
<i>F. culmorum</i>	3	8	6.55
<i>F. pseudograminearum</i>	0	5	2.98
<i>F. acuminatum</i>	13	24	22.02
<i>F. avenaceum</i>	10	22	19.05
<i>F. equiseti</i>	6	36	25.00
<i>Fusarium</i> spp.	6	9	8.93
<i>Cunninghamella</i> spp.	6	19	14.88
<i>Penicillium</i> spp.	1	0	0.60

In 2007, the emergence experiment was again conducted using stubs collected from postharvest wheat fields at 5 sites in Montana (Table 2.4). Adult emergence was greater than 80% from most sites, but emergence from stubs collected near Amsterdam was significantly lower than for other sites ( $\chi^2 = 14.87$ ,  $df = 4$ ,  $P < 0.05$ ). The percentage of fungal-colonized larvae ranged between 2.8% and 17.3% across sites, and Amsterdam had the greatest percentage of fungal colonization of larvae in diapause within stubs ( $\chi^2 = 17.84$ ,  $df = 4$ ,  $P < 0.05$ ). All sites had very low levels of parasitism by braconids. For other lethal factors, including drowning and mechanical damage, the percentage of larval mortality in the stubs collected near Amsterdam was significantly greater than for the other sites ( $\chi^2 = 67.09$ ,  $df = 4$ ,  $P < 0.05$ ) (Table 2.4). For fungal colonization in the field survey, significant difference between the sites was evident ( $F = 5.73$ ,  $df = 4$ ,  $P = 0.008$ ) (Figure 2.3). The mean percentage of colonized larvae was 20.0%, 15.0%, 12.5%, 11.9%, and 10.0% in Amsterdam, Choteau, Conrad, Havre, and Loring, respectively. The occurrence frequencies of fungal isolates from colonized larvae in the emergence experiment and field survey in 2007 are shown in Table 2.5 and Table 2.6 respectively. Members of the genus *Fusarium* were the dominant fungi isolated from colonized larval

cadavers within stubs collected from the 5 sites. Isolates of *Cunninghamella* spp. were also obtained from the colonized larvae.

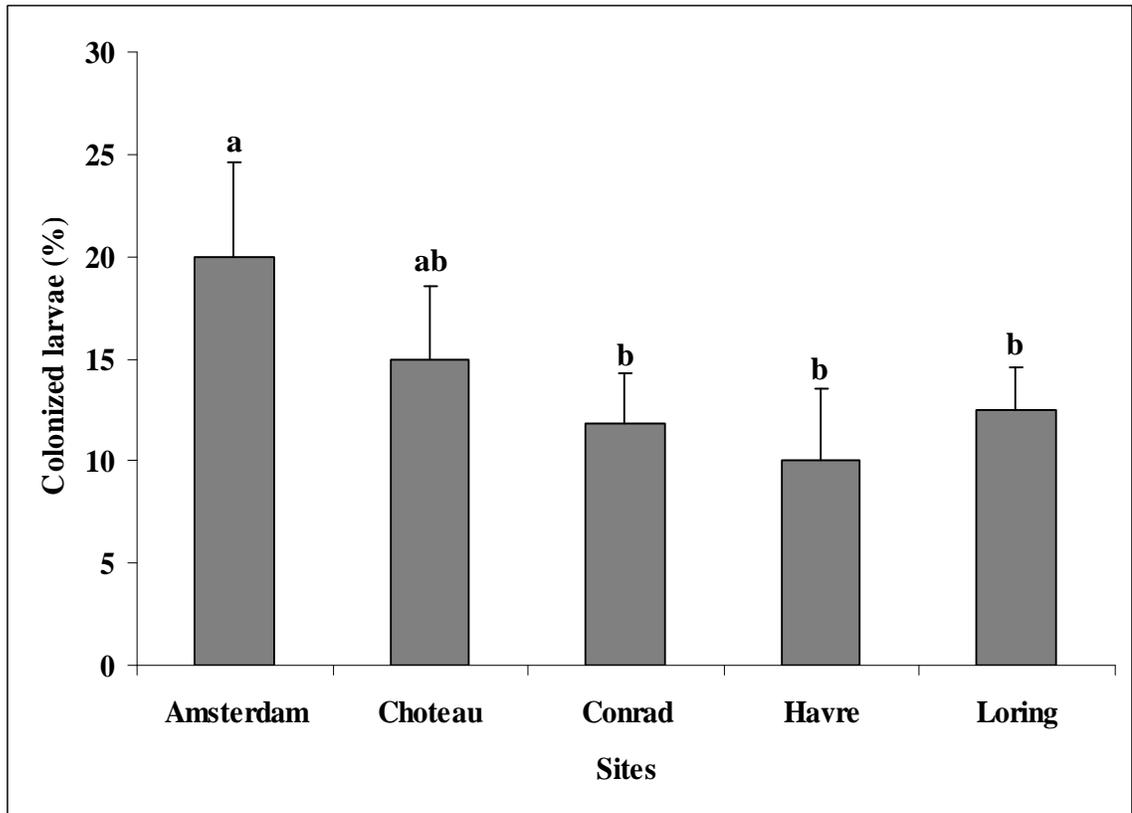


Figure 2.3. Percentage of fungal-colonized larvae within sawfly-cut stems collected from 5 sites in Montana in a field survey in 2007.

There were 160 sawfly-cut stems from each site; means indicated with different letters are significantly different (LSD = 4.10).

Table 2.4. Occurrence frequency (%) of mortality factors in sawfly-cut stems in 2007.

Overwinter status	Sites				
	Amsterdam	Choteau	Conrad	Havre	Loring
Emerged adults	52.21*	89.69	94.44	93.17	85.81
Colonized larvae	17.26*	5.84	2.78	3.60	9.46
Parasitized larvae	0	0	0.79	0.72	0
Other mortality factors	30.53*	4.47	1.98	2.52	4.73

The number of sawfly-cut stems from Amsterdam, Choteau, Conrad, Havre, and Loring was 226, 291, 252, 278, and 296, respectively.

\*Indicates significant difference between values in the row using a chi-square test ( $P < 0.05$ ).

Table 2.5. The number of fungal isolates from colonized larval cadavers within sawfly-cut stems in samples collected from locations across Montana in laboratory emergence experiments in 2007.

Fungal species	Sites					Overall colonization (%)
	Amsterdam	Choteau	Conrad	Havre	Loring	
<i>F. culmorum</i>	12	0	4	0	12	22.58
<i>F. pseudograminearum</i>	3	4	4	2	0	10.48
<i>F. acuminatum</i>	11	5	0	5	16	29.84
<i>F. avenaceum</i>	5	7	0	6	1	15.32
<i>F. equiseti</i>	8	0	0	0	0	6.45
<i>Fusarium</i> spp.	6	3	1	0	2	9.68
<i>Cunninghamella</i> spp.	1	0	0	0	3	3.23
<i>Penicillium</i> spp.	1	0	0	0	2	2.42

Table 2.6. The number of fungal isolates from colonized larval cadavers within sawfly-cut stems in samples collected from locations across Montana in field surveys in 2007.

Fungal species	Sites					Overall colonization (%)
	Amsterdam	Choteau	Conrad	Havre	Loring	
<i>F. culmorum</i>	6	4	13	0	3	17.11
<i>F. pseudograminearum</i>	2	5	7	4	0	11.84
<i>F. acuminatum</i>	16	9	0	6	7	25.00
<i>F. avenaceum</i>	6	7	9	10	0	21.05
<i>F. equiseti</i>	0	0	0	0	16	10.53
<i>Fusarium</i> spp.	2	4	0	2	3	7.24
<i>Cunninghamella</i> spp.	2	1	0	0	0	1.97
<i>Penicillium</i> spp.	0	3	0	3	2	5.26

## Discussion

The overwintering larval mortality of wheat stem sawfly could be evaluated by both laboratory emergence experiment and field survey, and both methods provided valuable information about naturally-occurring lethal factors in wheat fields. The field survey provided more specific information on mortality caused by fungal infection. However, some differences in larval mortality, especially the percentage of larval cadavers colonized by fungi, were obtained between the emergence experiment and field survey when considering stubs from the same site. The field survey usually indicated a greater percentage of colonized larvae than the emergence experiment. For example, the percentage of colonized larvae within stubs collected from Choteau in 2007 was 5.8% in the emergence experiment, and was 15.0% for the field survey (Table 2.4 and Figure 2.3). The variations in these two methods result from differences in processing the stubs after they were collected from the wheat field. For the field survey, all stubs were dissected immediately in the laboratory, and the larval mortality was classified and recorded without paying special attention to the superficial quality of the stubs. For the emergence experiment, only intact stubs showing no obvious mechanical damage and no evidence of fungal attack were selected for testing, to better increase our understanding about mortality through to adult emergence, which could also explain the low percentage of colonized larvae in the emergence experiment for 2005 (Table 2.1). The field survey more accurately reflected the actual colonization of larval cadavers in the field, while the emergence experiment provided more information about the relative contributions of

various lethal factors to the overall survival of the adults. There could be some overlaps of the percentage of colonized larvae for the emergence experiment and the field survey, and the actual percentage of colonized larvae in the field should be greater than that shown in the field survey and less than the combined total for both. For example, the best estimate for the percentage of colonized larvae from Choteau in 2007 should be lie between 15.0% and 20.8% (Table 2.4 and Figure 2.3).

In this study, fungal colonization, mainly by *Fusarium* spp., probably indicates the most important lethal factor for larvae in diapause within stubs during the winter, and could account for 10 to 20 percent of the overall mortality depending on the environmental factors. In another experimental study, the *Fusarium* spp. isolates (one of each from *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum*) obtained from colonized larval cadavers of wheat stem sawfly were evaluated against the healthy overwintering sawfly larvae within stubs in a postharvest environment in both growth chamber and field experiments over two consecutive winters. The tested *Fusarium* spp. isolates could successfully colonize the stubs, and cause twenty to sixty percent mortality in diapausing larvae (Sun et al. Chapter three, In preparation). Other mortality factors impacting overwintering larvae were parasitoids, predators, drowning, and mechanical damage. However, the mortality due to parasitoids and predators is limited because both are relatively unable to access the sawfly larvae contained within stubs for the brief postharvest interval before temperatures decrease to limit insect activity (Table 2.1 and Table 2.4). Noticeable mortality due to drowning occurred only in stubs collected near Amsterdam in 2007, probably due to standing water in the early spring, which also may

have increased the percentage of colonized larvae compared to other sites because immersion may have compromised the immunity of living larvae and facilitated subsequent fungal infection, or may have suffocated the larvae and allowed saprophytic utilization (Table 2.4).

These *Fusarium* species are the dominant fungi isolated from larval cadavers for both emergence experiments and field surveys over three years. *Fusarium* species are good competitive saprophytes colonizing wheat straw, and the straw penetration rate is closely correlated with the rate of cellulolysis, suggesting that the speed of penetration depends on the rate of enzymatic degradation of the portion of the cell wall surrounding the apices of the penetrating hyphae. In nitrogen deficient situations, *Fusarium* species are able to produce chitinases to recycle nitrogen from chitin and other nitrogen bearing compounds from wheat straw (Garret 1975a and 1975b). *Fusarium* species also produce macroconidia and chlamydospores to persist over an atypically broad range of temperature and moisture levels for years (Wilcock and Megan 2001). The ability to colonize wheat straw and residue under dry, cold winter weather conditions distinguishes *Fusarium* species from other entomogenous microorganisms that utilize sawfly larvae. The saprophytic traits enable *Fusarium* species to attack the cryptic larvae inside hibernacula within the protective stubs. Highly-degraded stubs compromise the innate immunity of overwintering sawfly larvae in the field under Montana weather conditions, and this makes the overwintering larvae more susceptible to subsequent infection by the *Fusarium* species. In this study, the most frequently isolated *Fusarium* species were *F. culmorum*, *F. pseudograminearum*, *F. acuminatum*, *F. avenaceum*, and *F. equiseti*. The

frequency of occurrence of these species varied across sites and years, which could have resulted from the interactions between environmental factors, wheat varieties, and soil types (Table 2.3, Table 2.5, and Table 2.6).

In summary, the overwintering larval mortality of wheat stem sawfly were evaluated by both emergence experiments and field surveys, both of which indicate that fungal colonization of stubs by *Fusarium* spp. occurs frequently in postharvest wheat fields, and this facilitates entomopathogenicity to overwintering larvae. Complexes of *Fusarium* spp. were the dominant fungi isolated from colonized larvae across multiple sites and years. Further research exploring the intricate interactions between environmental and biotic factors in this system will increase our understanding of this antagonistic relationship between ubiquitous *Fusarium* spp. and immature wheat stem sawflies. This information can be incorporated into postharvest management of the wheat fields that are the overwintering reservoirs for wheat stem sawfly populations.

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## CHAPTER THREE

OVERWINTER COLONIZATION OF DIAPAUSING LARVAE OF WHEAT STEM  
SAWFLY (HYMENOPTERA: CEPHIDAE) WITHIN STEM HIBERNACULA BY  
*FUSARIUM* SPP. ISOLATESAbstract

Single isolates of three *Fusarium* spp., including *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum*, obtained from fungal-colonized larval cadavers of wheat stem sawfly, were evaluated against healthy overwintering larvae within sawfly-cut stems in a postharvest environment by using growth chamber and field experiments over two consecutive winters. These *Fusarium* isolates had lethal impacts on the overwintering larvae, and could cause twenty to sixty percent larval mortality, depending on the environmental conditions. The *F. pseudograminearum* and *F. culmorum* isolates caused greater decomposition in sawfly-cut stems and also produced greater mortality in overwintering larvae than the *F. acuminatum* isolate. Strong positive correlations between the decomposition rating and larval mortality reflect the interchangeability of the *Fusarium* isolates as saprophytes on wheat stubble and as pathogens of sawfly larvae. As ubiquitous soil fungi, *Fusarium* spp. are annually impacting larval populations of the wheat stem sawflies that are in overwinter diapause in wheat fields.

### Introduction

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is the most destructive insect pest to both winter wheat and spring wheat production in the northern Great Plains (Weiss and Morrill 1992, Morrill et al. 1998). Yield loss estimates range from 5-15% decrease in seed weight, depending on variety and year (Morrill et al. 1992). However, a more conspicuous loss is the lodging of the wheat stems caused by larval cutting just before harvest, which results in unrecoverable mature stems. Yield loss can reach 50 kg/ha (Beres et al. 2007) at frequently encountered levels of lodging. Annual losses in all impacted wheat production regions may exceed 100 million dollars for wheat at 2003 prices (Hartel et al. 2003, Weaver et al. 2004), or approximately 250 million dollars at the current prices. Adults emerge from the wheat residue remaining after the harvest of the previous year. Emergence of adults usually extends from late May through July in the northern Great Plains (Criddle 1915, Wallace and McNeal 1966, Weiss et al. 1990). Females fly to nearby hosts and lay one or more eggs in each stem (Holmes and Peterson 1960). After hatch, the larva feeds on plant tissues in the stem lumen for a month or longer, and cuts an internal v-shaped notch at the base of the stem at plant maturity, coincident with harvest (Criddle 1917). The girdling sites are generally less than 25 mm above ground level, and the larvae will produce a long, thin, and transparent tube, called a 'hibernaculum', below the girdling site in order to survive the extreme winter weather conditions. The larva overwinters in diapause in hibernaculum within the sawfly-cut stem, which is called a 'stub' (Morrill et al. 1998). An eight to nine

months diapause in the wheat residue at or below soil surface is obligatory, and metamorphosis begins as the soil warms the following spring (Wallace and McNeal 1966, Holmes 1975).

The life cycle of the wheat stem sawfly explains the difficulty in controlling its population because all immature stages are protected within wheat stems. However, the overwintering interval in the stubs exposes larvae to invasion by soil microorganisms. Previous research reported fungal-colonized larval cadavers inside stubs collected in the postharvest wheat fields. Isolates of *Fusarium* spp., including *F. graminearum* Schwabe Gr1 (syn. *pseudograminearum*), *F. culmorum* (W. G. Smith) Sacc., *F. acuminatum* Ell. & Ev. Sensu Gordon, *F. avenaceum* (Fr.) Sacc., and *F. equiseti* (Corda) Sacc. Sensu Gordon, were isolated from these larval cadavers (Wenda-Piesik et al. 2006; In preparation). These *Fusarium* spp. are well-known causative agents of crown and root rot, as well as head blight, in wheat growing areas in Montana (Hogg et al. 2007). They are also vigorous saprophytic colonizers of wheat straw in the field (Walker 1941). Pathogenicity of these *Fusarium* isolates to diapausing larvae was confirmed in laboratory bioassays, and more than 80% larval mortality was observed after 13 days (Wenda-Piesik et al. 2006; In preparation). The *Fusarium* isolates also caused larval mortality within stems of growing spring wheat (Wenda-Piesik et al. In preparation) and growing winter wheat plants (Sun et al. Chapter four, In preparation). There are no previous studies of postharvest effects of *Fusarium* spp. on overwintering larvae of wheat stem sawfly inside stubs. In this paper, we report on pathogenic effects of the *Fusarium*

isolates on the overwintering larvae in growth chamber and field experiments over two consecutive winters.

## Materials and Methods

### *Fusarium* Inoculum

Single isolates of three *Fusarium* spp., including *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum*, were isolated from wheat stem sawfly larval cadavers that were collected in postharvest wheat fields in Pondera and Broadwater Counties, Montana, in the fall of 2001 and 2002. The *Fusarium* isolates were identified by the *Fusarium* Research Center at Pennsylvania State University, and a mycological specimen of each isolate was deposited at this facility (Wenda-Piesik et al. 2006; In preparation). Single-spore cultures were rejuvenated on potato dextrose agar (PDA) media at 23 °C for 7 days. Aerial mycelia were harvested by superficially scraping them from the culture surface with a scalpel and were used to inoculate autoclaved oat kernels under sterile conditions. The cultures were incubated for 3 weeks at 23 °C. Colonized oat kernels were transferred to a screen-bottomed tray and air-dried in a ventilated hood for 3 days before sieving to obtain uniform-sized inoculum for experiments.

### Collection of Larvae in Diapause

Sawfly infested stubble were collected from postharvest wheat fields near Amsterdam, Montana, during October of 2005 and again in 2006. The heavily-infested susceptible hollow-stem wheat varieties were ‘McNeal’ in 2005 and ‘Reeder’ in 2006. Stubs were readily sorted by identifying precise, uniform cutting, and also by recognizing

the frass plugs sealing the tops of the cut stems. Only intact stubs with no obvious mechanical damage and no evidence of fungal attack were selected for experiments.

#### Growth Chamber Experiments

These were conducted in the Plant Growth Center at Montana State University in mid-October of 2005 and 2006. For each fungal treatment, 2.5 grams of inoculum prepared from the *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* isolates was thoroughly mixed with 250 grams of soil in a 20 × 10 × 10 cm soil tray. In each treatment ten intact stubs were buried at 5 mm below the soil surface, while for the control the stubs were buried in soil only. There were four replications of each treatment. Bozeman silt loam soil was collected from field sites where related field experiments were carried out at the Post Experimental Farm of Montana State University. The soil trays containing the stubs were covered with plastic wrap and incubated at 4 °C with 15% soil moisture for 6 months, which simulated field conditions during the winter at the soil collection sites. After incubation for six months, the stubs were removed from the soil trays and processed in the laboratory. This occurred in mid-April of 2006 and 2007.

In the laboratory, each stub was scored on the extent of discoloration caused by the *Fusarium* isolates as: 1 = slight, where the stubs had little or no discoloration; 2 = moderate, where the stubs had an intermediate purplish brown color; and 3 = severe, where the stubs were very dark, typically reddish brown. A decomposition rating for each *Fusarium* treatment was based upon the number of stubs in each classification category and the numerical value assigned to the respective category, which yields a weighted

average based on the number of stubs showing varying degrees of discoloration. The decomposition rating (DR) percentage is calculated as follows:

$$\text{decomposition rating (DR) \%} = 100(n_i D_i) / ND_{max},$$

in which  $n_i$  is the number of stubs of the  $i$ th category,  $D_i$  is the numerical value of the  $i$ th category,  $N$  is the total number of stubs in the sample, and  $D_{max}$  is the maximum category value, which is 3 (Grey and Mathre 1987).

The percentage of larval mortality for each treatment was determined by counting the number of the dead larvae and living larvae found in the stubs during dissection. Other factors infrequently causing larval mortality, such as the extremely rare attack by idiobiont parasitoids in the stubs, were excluded because they only accounted for a tiny proportion of the observed larval mortality. Infection by the *Fusarium* isolates was confirmed by the presence of pink, tan, or white typical fungal mycelia on the surface of sawfly larval cadavers, and confirmed by subsequent re-isolation of the *Fusarium* spp. from the larval cadavers by using single-conidia technique on both potato dextrose agar and carnation leaf agar slants for culture (Nelson et al. 1983). The larval mortality and stub decomposition in the control treatments were mainly due to naturally-occurring *Fusarium* spp., including *F. culmorum*, *F. pseudograminearum*, *F. acuminatum*, *F. avenaceum*, and *F. equiseti*.

### Field Experiments

These were conducted in postharvest winter wheat fields at the Post Experimental Farm, Montana State University, in mid-October of 2005 and 2006. The plots were at different locations at the Post Experimental Farm each year. The plots were established

where the winter wheat variety ‘BigSky’ had been planted concurrently with inoculum of the *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* isolates at a rate of 10 grams of inoculum per 3-meter row in the previous year. At planting, there were 4 rows of wheat plants in each 3 × 1.2 meter plot, and identical control plots were sown without inoculum. These treatments were arranged in a randomized block design and replicated four times. These plots were harvested at the end of the summer. The postharvest plots for these treatments and the associated controls were used to subsequently assess overwinter pathogenicity of ‘residual inoculum’, soil containing infected subsurface wheat residue, to sawfly larvae. Stubs were added to these established field plots by burying them at 5 mm below the soil surface in the postharvest rows containing stubs at a rate of sixty per meter to a portion of the established plot, resulting in a 0.3-meter replicate. Stubs were also added to the former control plots at the same rate to serve as the control. The *Fusarium* inoculum was also added to the former control plots to assess overwinter pathogenicity of ‘supplemented inoculum’, *Fusarium* colonized oats kernels, to sawfly larvae. These supplemented replicates were established by burying twenty stubs, plus five grams of supplemented inoculum per 0.3-meter at 5 mm below the soil surface into the rows of wheat stubble. The inocula were mixed thoroughly with soil at a ratio of 1:100 before the stubs were buried. In addition, a second control was prepared using ‘thiophanate-methyl plus mancozeb’ fungicide; 2.5 grams of fungicide was mixed thoroughly with 500 grams of soil before it was buried with twenty stubs into a 0.3-meter row containing wheat stubble, also in the former control plots. The fungicide was added to reduce infection by endemic microorganisms in the field. Fungicide-treated controls

were included for specific comparison to the controls that were not supplemented with inoculum after harvest.

Each year, all stubs were held in the field for six months under naturally-occurring winter conditions. The conditions were an overall average temperature of 1.6 °C (monthly range between -5.0 °C and 8.1 °C), an overall average relative humidity of 60.2% (monthly range between 51.9% and 68.0%), with total precipitation of 33 cm at the location of the experiments for both years. The treated stubs were collected from the field sites as the soil began warming in mid-April of 2006 and 2007. In the laboratory, the decomposition rating and larval mortality were calculated as they were for the growth chamber experiments.

### Statistical Analysis

Arcsine square-root transformations were performed on all percentage data for larval mortality and decomposition rating before analysis. A split-plot analysis of variance (ANOVA), with year as the main plot, was used to examine the effects of *Fusarium* treatments on the larval mortality and decomposition rating in both growth chamber and field experiments (PROC MIXED, SAS Institute 2001). Data for both years (2006 and 2007) were combined for analysis if year showed no significant effect. Data of each year were analyzed separately by ANOVA if year showed significant effect (PROC MIXED, SAS Institute 2001). Multiple comparisons were made between the *Fusarium* treatments by separating the means using the Fisher least significance difference (LSD) at  $\alpha = 0.05$ . The relationship between larval mortality and disease rating was assessed by correlation analysis (PROC CORR, SAS Institute, 2001) and the Pearson correlation

coefficient was used to assign significance at  $P < 0.05$ . Student's t-test was used to make pairwise comparisons between the control and the fungicide-treated control treatments (PROC TTEST, SAS Institute 2001).

## Results

### Growth Chamber Experiments

In these experiments in 2006 and 2007, all tested *Fusarium* isolates were pathogenic to overwintering larvae when incubated at 4 °C with 15% soil moisture for 6 months ( $F = 54.98$ ,  $df = 3$ ,  $P < 0.001$ ) (Figure 3.1). The mean larval mortality did not differ significantly between years ( $F = 0.81$ ,  $df = 1$ ,  $P = 0.434$ ), and the interaction between year and treatment was not significant ( $F = 0.94$ ,  $df = 3$ ,  $P = 0.441$ ). The mean larval mortality in the *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* treatments was 43.0%, 44.3%, and 28.4%, respectively. Mean larval mortality in the control was 7.2%, which was significantly less than that for the *Fusarium* treatments by multiple comparisons. The *F. pseudograminearum* and *F. culmorum* isolates produced significantly greater lethal effects in the larvae than the *F. acuminatum* isolate, while there was no significant difference between the *F. pseudograminearum* and *F. culmorum* isolates in pathogenicity to the larvae (Figure 3.1).

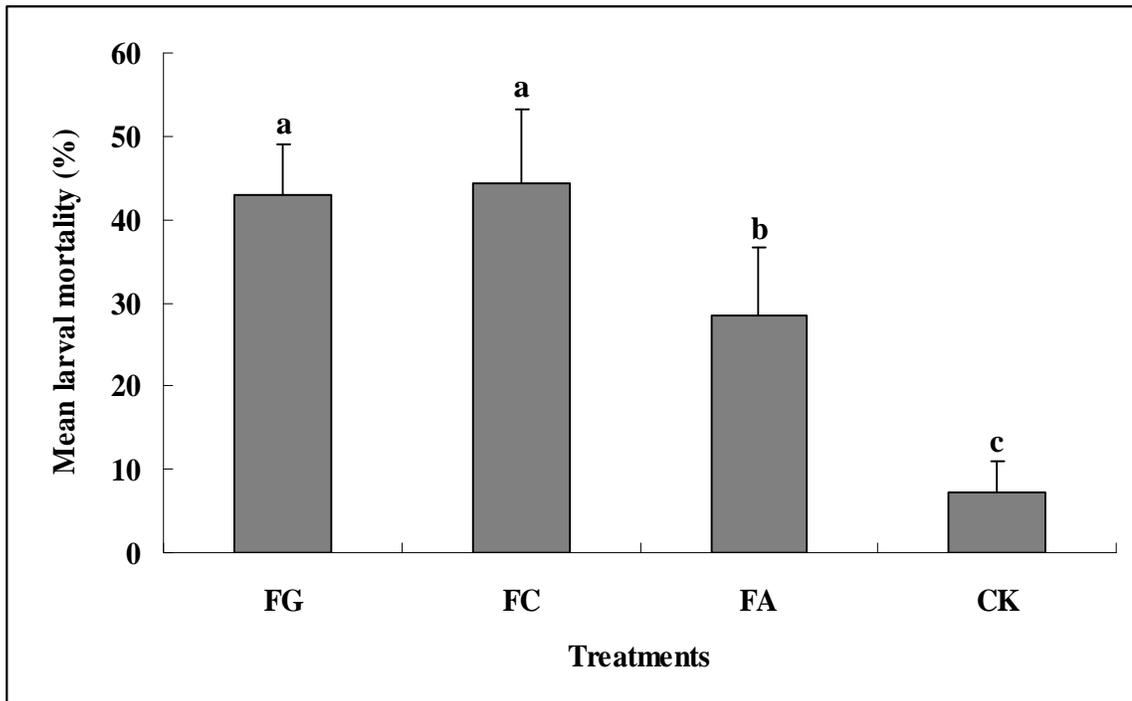


Figure 3.1. Mean mortality of diapausing larval wheat stem sawflies in *Fusarium* and control treatments in growth chamber experiments in 2006 and 2007. FG - *F. pseudograminearum*, FC - *F. culmorum*, FA - *F. acuminatum*, and CK - control. Each bar represents the combined data of treatments for both 2006 and 2007; the letters a, b and c indicate the significant differences between the means for the treatments; means are separated by LSD = 5.12 ( $\alpha = 0.05$ ).

The rating for decomposition caused by the *Fusarium* isolates reflected a different capability for colonizing stubs. Significant differences were noted in decomposition rating among treatments (2006 -  $F = 17.84$ ,  $df = 3$ ,  $P = 0.0004$ ; 2007 -  $F = 267.79$ ,  $df = 3$ ,  $P < 0.001$ ) (Figure 3.2). The mean decomposition rating differed among years ( $F = 157.20$ ,  $df = 1$ ,  $P = 0.001$ ), and the interaction between year and treatment was also significant ( $F = 8.83$ ,  $df = 3$ ,  $P = 0.001$ ). The mean decomposition rating of *Fusarium* treatments was higher in 2007 than that in 2006, but a different pattern of stub colonization caused by the *Fusarium* isolates was shown for both 2006 and 2007, which

explains the significant year effect and interaction between year and treatment. The mean decomposition rating in the *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* treatments was 59.3%, 59.6%, and 49.9%, respectively in 2006, and 70.8%, 74.5%, and 66.5%, respectively in 2007. The mean decomposition rating in the control was 43.2% in 2006 and 47.3% in 2007 respectively, which was significantly lower than that of the *Fusarium* treatments. The *F. pseudograminearum* and *F. culmorum* isolates caused greater decomposition rating than the *F. acuminatum* isolate in the growth chamber experiments for both years, with no significant difference in the decomposition ratings for the *F. pseudograminearum* and *F. culmorum* isolates (Figure 3.2). Data for larval mortality and decomposition rating in the *Fusarium* treatments showed similar patterns, and significant positive correlations in the growth chamber experiments were statistically confirmed for 2006 ( $n = 16$ ,  $r = 0.929$ ,  $P < 0.001$ ) and for 2007 ( $n = 16$ ,  $r = 0.960$ ,  $P < 0.001$ ) respectively (Figure 3.3).

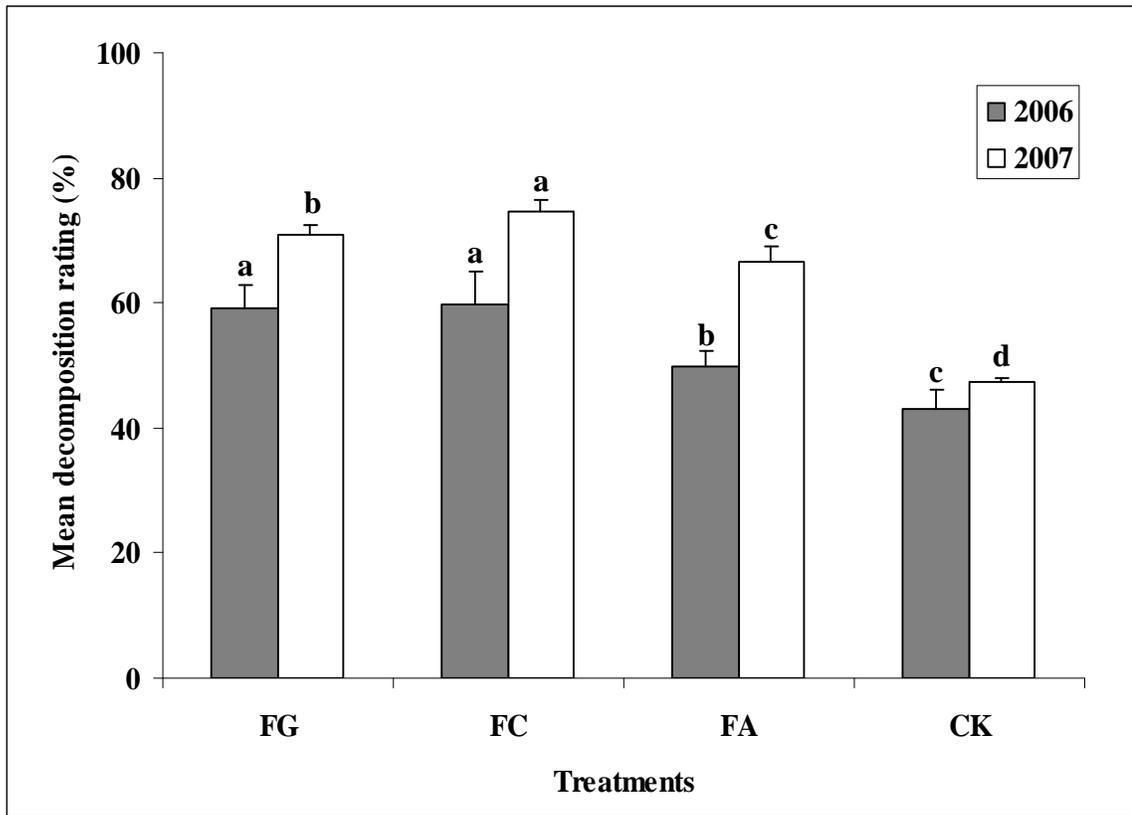


Figure 3.2. Mean decomposition rating of sawfly-cut stems in *Fusarium* and control treatments in growth chamber experiments in 2006 and 2007. FG - *F. pseudograminearum*, FC - *F. culmorum*, FA - *F. acuminatum*, and CK - control. Each bar represents the data for treatments in 2006 or 2007; the letters a, b and c indicate the significant differences between the means for the treatments in each year; means are separated by LSD = 3.47 in 2006 and LSD = 1.41 in 2007 ( $\alpha = 0.05$ ).

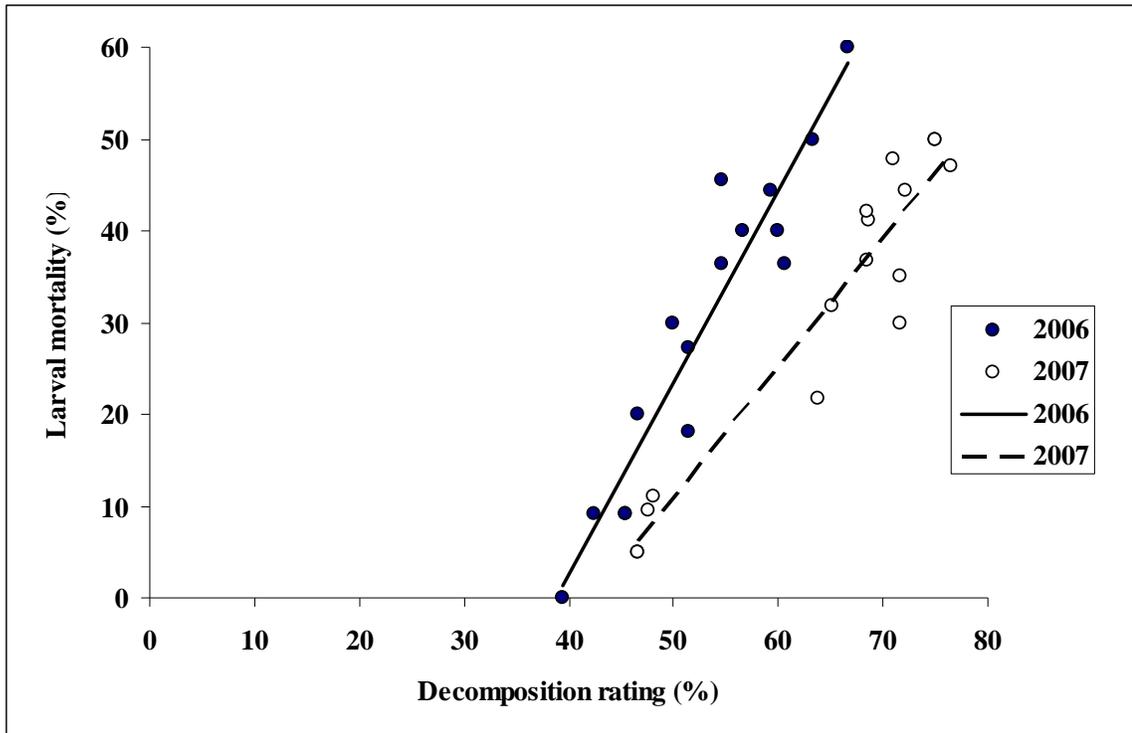


Figure 3.3. Correlation between larval mortality and decomposition rating in growth chamber experiments in 2006 and 2007.

### Field Experiments

In 2006 and 2007, all the tested *Fusarium* isolates showed pathogenic effects to the overwintering larvae under the winter conditions in Montana after 6 months of incubation, and significant differences were noted in larval mortality among *Fusarium* treatments ( $F = 31.48$ ,  $df = 3$ ,  $P < 0.001$ ) (Figure 3.4). The mean larval mortality did not differ significantly between years ( $F = 0.25$ ,  $df = 1$ ,  $P = 0.649$ ), and the interaction between year and treatment was not significant ( $F = 1.65$ ,  $df = 3$ ,  $P = 0.163$ ). The residual *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* inocula caused 25.4%, 23.0%, and 21.9% mean larval mortality respectively, which was significantly higher than that of

the control with 11.7% mean larval mortality. For the residual inoculum treatments, there was no significant difference in larval mortality among the *Fusarium* isolates. The mean larval mortality in the supplemented *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* inocula treatments was 48.9%, 44.6%, and 33.6% respectively, which was significantly higher than that of both residual inoculum and control treatments. For the supplemented inoculum treatments, the *F. pseudograminearum* and *F. culmorum* isolates caused significantly greater lethal effects in larvae than the *F. acuminatum* isolate, while there was no significant difference between the *F. pseudograminearum* and *F. culmorum* isolates for larval mortality (Figure 3.4).

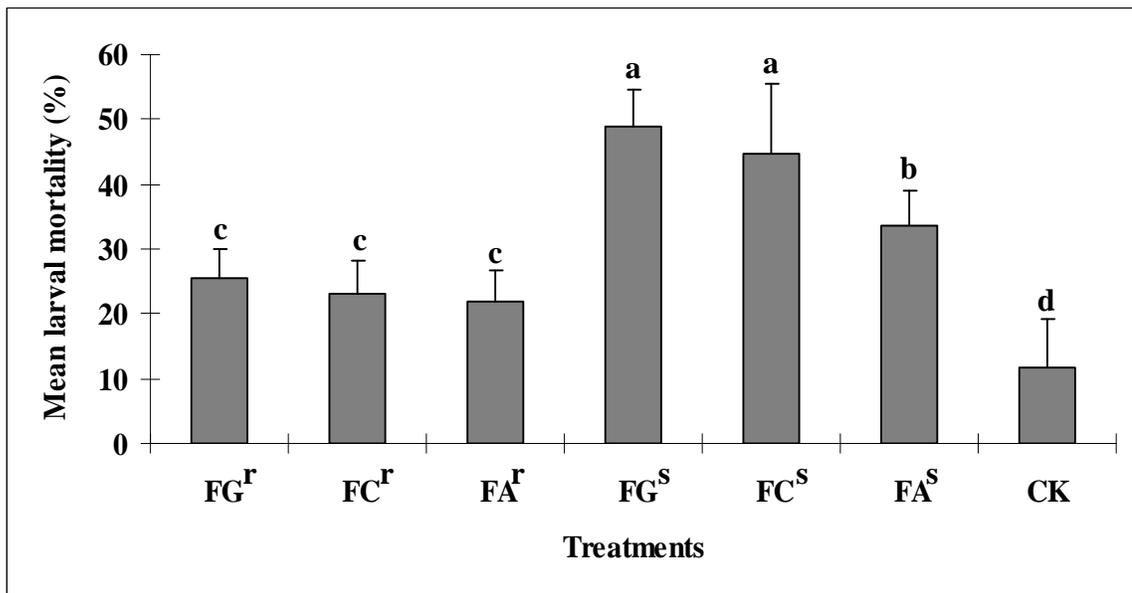


Figure 3.4. Mean mortality of larval wheat stem sawflies in *Fusarium* and control treatments in field experiments in 2006 and 2007. FG - *F. pseudograminearum*, FC - *F. culmorum*, FA - *F. acuminatum*, and CK - control; r - residual inoculum, s - supplemented inoculum.

Each bar represents the combined data for treatments in 2006 and 2007; the letters a, b, c, and d indicate significant differences between the means for the treatments; means are separated by LSD = 5.11 ( $\alpha = 0.05$ ).

In the field experiments in 2006 and 2007, all the tested *Fusarium* isolates displayed colonization capabilities for stubs under winter conditions in Montana over a 6 month exposure period, and significant differences were noted in the decomposition ratings among *Fusarium* treatments (2006 -  $F = 17.32$ ,  $df = 3$ ,  $P < 0.001$ ; 2007 -  $F = 29.89$ ,  $df = 3$ ,  $P < 0.001$ ) (Figure 3.5). The mean decomposition rating varied among years ( $F = 75.55$ ,  $df = 1$ ,  $P = 0.003$ ), and the interaction between year and treatment was also significant ( $F = 3.03$ ,  $df = 3$ ,  $P = 0.017$ ). There was an obvious increase in the mean decomposition rating among *Fusarium* treatments between 2006 and 2007, but the different trend of stub colonization caused by the *Fusarium* isolates was evident for 2006 and 2007, which explains the significant year effect and interaction between year and treatment. For the residual inoculum treatments, the mean decomposition rating of the *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* treatments was 52.7%, 48.3%, and 47.2%, respectively in 2006, and 60.8%, 59.7%, and 58.1%, respectively in 2007. The mean decomposition rating of the control treatment was 45.8% in 2006 and 53.4% in 2007 respectively. There were significant differences in decomposition rating between the residual inoculum and control treatments in 2007, but not in 2006. For the supplemented inoculum treatments, the mean disease rating in the *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* treatments was 67.2%, 66.3%, and 56.3%, respectively in 2006 and 72.0%, 69.3%, and 67.4%, respectively in 2007, which was significantly higher than that for both the residual inoculum and control treatments. The *F. pseudograminearum* and *F. culmorum* isolates had greater colonization capabilities for the stubs than the *F. acuminatum* isolate in both 2006 and

2007, while no significant difference was statistically evident between the *F. culmorum* and *F. acuminatum* isolates in 2006 or 2007 (Figure 3.5). A strong positive correlation between larval mortality and decomposition rating in the treatments was statistically evident in the field experiments in 2006 ( $n = 28$ ,  $r = 0.891$ ,  $P < 0.001$ ) and 2007 ( $n = 28$ ,  $r = 0.846$ ,  $P < 0.001$ ) respectively (Figure 3.6).

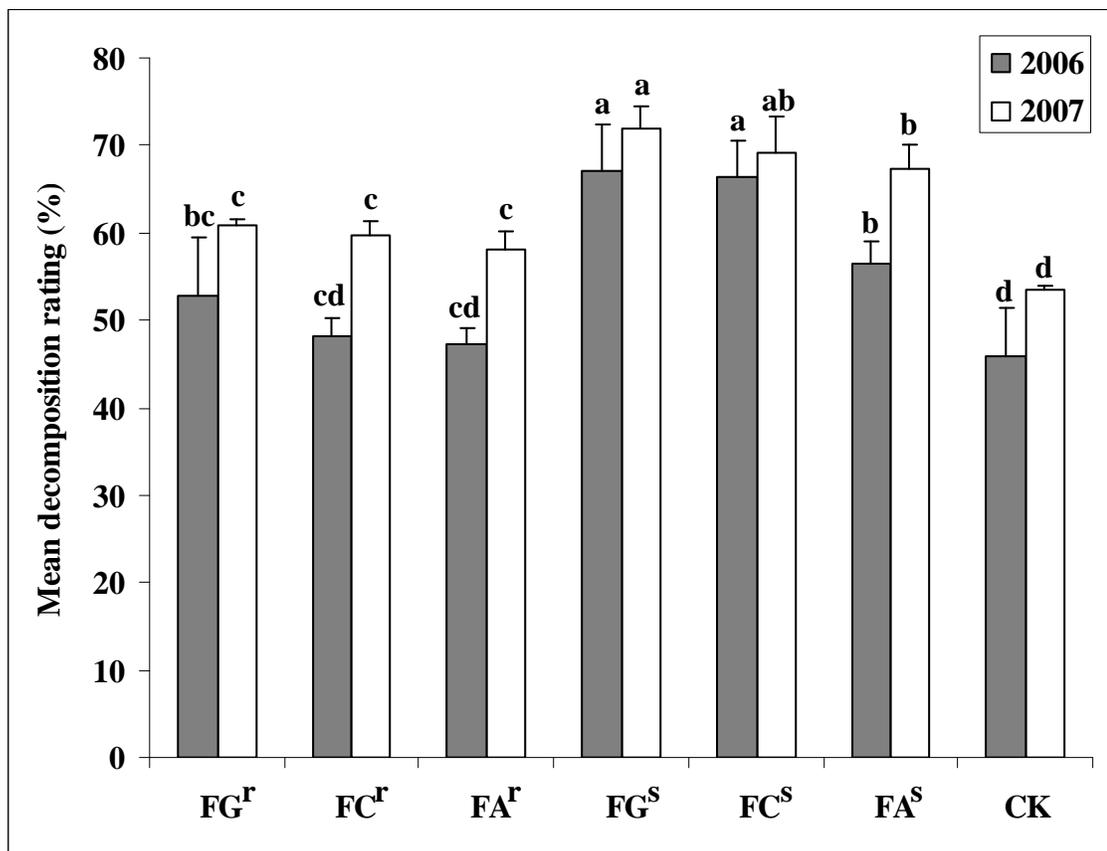


Figure 3.5. Mean decomposition rating of sawfly-cut stems in *Fusarium* and control treatments in field experiments in 2006 and 2007. FG - *F. pseudograminearum*, FC - *F. culmorum*, FA - *F. acuminatum*, and CK - control; r - residual inoculum, s - supplemented inoculum.

Each bar represents the individual data for treatments in 2006 or 2007; the letters a, b and c indicate the significant differences between the means for the treatments in each year; means are separated by  $LSD = 3.71$  in 2006 and by  $LSD = 2.25$  in 2007 ( $\alpha = 0.05$ ).

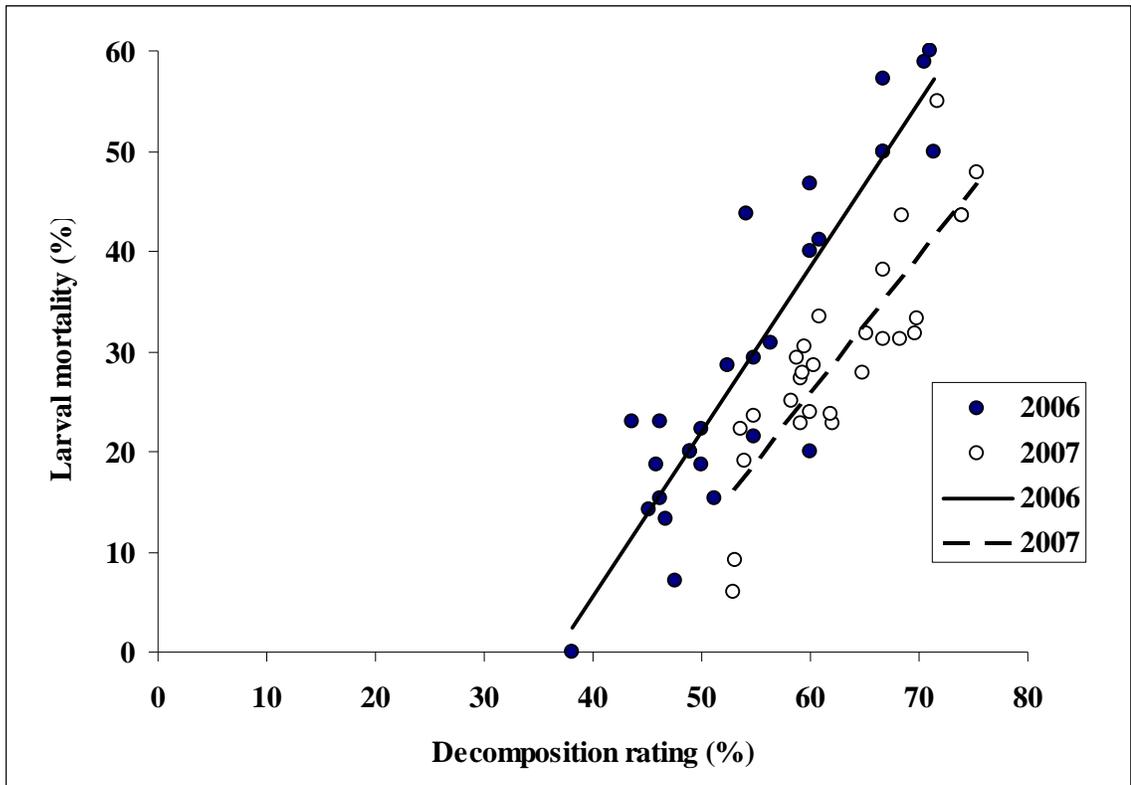


Figure 3.6. Correlation between larval mortality and decomposition rating in field experiments in 2006 and 2007.

Table 3.1. Mortality of larval wheat stem sawflies and decomposition rating of sawfly-cut stems in the fungicide-treated and control treatments in 2006 and 2007.

Treatments	Larval mortality (%)		Decomposition rating (%)	
	(Mean $\pm$ SE)		(Mean $\pm$ SE)	
	2006	2007	2006	2007
Fungicide-treated control	5.1 $\pm$ 3.3	6.3 $\pm$ 3.0	42.9 $\pm$ 2.6	44.4 $\pm$ 2.7
Control	9.5 $\pm$ 7.4	14.1 $\pm$ 7.8	45.8 $\pm$ 5.6	53.4 $\pm$ 0.5

The fungicide protected sawfly larvae and stubs from attack by naturally-occurring *Fusarium* spp. in the control plots. Although there was no significant difference in mean larval mortality between the fungicide-treated control and the control treatments in 2006 ( $t = 0.45$ ,  $df = 6$ ,  $P = 0.667$ ) and 2007 ( $t = 1.85$ ,  $df = 6$ ,  $P = 0.113$ ), the mean

larval mortality in the fungicide-treated control was 45.9% lower in 2006 and 55.3% lower in 2007, respectively, compared to the control treatments (Table 3.1). However, significant differences in the decomposition rating for stubs were observed between the fungicide-treated control and the control treatments in 2006 ( $t = 2.77$ ,  $df = 6$ ,  $P = 0.032$ ) and 2007 ( $t = 6.99$ ,  $df = 6$ ,  $P = 0.0004$ ). The fungicide reduced the mean decomposition rating in the fungicide-treated control treatments by 6.2% in 2006 and 16.9% in 2007 respectively, compared to the control treatments (Table 3.1).

### Discussion

Wheat stem sawfly larvae overwinter in wheat stubble for eight to nine months at or below the soil surface, which makes this prolonged immobile stage a major period of vulnerability that has significant potential for impact on developing populations. There are several challenges to be overcome to colonize overwintering cryptic larvae. The first barrier encountered before accessing larvae in hibernacula is the protection provided by the external wheat stem. This material is a highly lignified cellulose complex, which denies the access of most entomogenous microorganisms. An effective entomopathogen needs to conquer both the external protection of the wheat stubble and the innate immunity of the larvae. *Fusarium* spp. are well-known competitive saprophytes colonizing wheat straw, and the rate of straw penetration is closely correlated with the speed of cellulolysis. The speed of penetration depends on rate of enzymatic degradation of the material around the apices of penetrating hyphae. In nitrogen deficient situations, *Fusarium* spp. are able to produce chitinases to recycle nitrogen from chitin and other

nitrogen bearing compounds in wheat straw (Garret 1975a and 1975b). A variety of *Fusarium* spp. are frequently isolated from wheat straw, these species included *F. culmorum*, *F. pseudograminearum*, *F. acuminatum*, *F. graminearum*, *F. avenaceum*, and *F. equiseti* (Klaasen et al. 1991, Luque et al. 2005), and these species were occasionally isolated from colonized materials in our untreated plots. The ability to colonize wheat stubble distinguishes *Fusarium* spp. from other entomogenous microorganisms attacking sawfly immatures (Wenda-Piesik et al. In preparation) because the saprophytic characteristics enable the breakdown of the protective stubs, and allow the attack of the overwintering larvae.

In this study, the *F. pseudograminearum* and *F. culmorum* isolates caused greater larval mortality than the *F. acuminatum* isolate, and they also appeared to more aggressively colonize stubs as saprophytes. This might make these isolates more pathogenic to larvae after penetrating the stubs, or the greater ability to decompose the cellulose and lignin complexes of wheat stubble compromises the innate immunity of overwintering larvae in the field in Montana, and results in greater susceptibility to subsequent infection. These *Fusarium* isolates were also found to be able to colonize wheat stems of growing plants as plant pathogens, and attack developing larvae inside the stems. The *F. pseudograminearum* and *F. culmorum* isolates also caused greater sawfly larval mortality and stem disease reactions than the *F. acuminatum* isolate in both spring and winter wheat trials (Wenda-Piesik et al. In preparation, Sun et al. Chapter four; In preparation).

*Fusarium* spp. produce macroconidia and chlamydospores that facilitate survival and maintain biological activity over an unusually wide range of temperature and moisture levels for years (Wilcock and Megan 2001). Winters in the area that the wheat stem sawfly inhabits are characterized by fluctuating temperatures with low humidity and precipitation (Morrill et al. 2001). The larval mortality and decomposition ratings in this study, especially for the previously established treatments, support the fact that those *Fusarium* isolates could adapt to cold and dry winter conditions and still be saprophytic on stubs and pathogenic to larvae within stubs (Figure 3.4 and Figure 3.5). The *Fusarium* isolates showed relatively consistent impacts on the sawfly larvae in both growth chamber and field experiments across two years. Occasional yearly variation in decomposition ratings could have resulted from the different wheat varieties from which the stubs were collected, from different field locations, or possibly from different weather conditions over the two winter seasons. Interestingly, we conducted a postharvest wheat field survey of mortality in overwintering larvae by collecting stubs from five locations of Montana in March, 2007. All surveyed locations had more than 10% mortality in the diapausing larvae, and with more than 90% caused by *Fusarium* spp., including *F. culmorum*, *F. pseudograminearum*, *F. acuminatum*, *F. avenaceum*, and *F. equiseti* (Sun et al. Chapter one; In preparation). The marginally reduced levels of sawfly larval mortality and the reduced stub decomposition rating in the fungicide-treated control, compared to the control treatments for this study, also supported the fact that naturally-occurring *Fusarium* spp. were the major factors causing overwintering larval mortality,

and their ability to colonize stubs and attack larvae can be inhibited by the presence of the fungicide (Table 3.1).

In summary, the *Fusarium* isolates in this study produced lethal impacts on overwintering larvae under either manipulated or natural conditions, and could account for twenty to sixty percent annual population decreases in the field, depending on environmental and biotic factors. The *F. pseudograminearum* and *F. culmorum* isolates caused greater saprophytic decomposition of stubs and greater mortality of overwintering larvae than the *F. acuminatum* isolate. The strong correlations between decomposition rating and larval mortality reflect plasticity of the *Fusarium* isolates as saprophytes on wheat residue and as subsequent pathogens on sawfly larvae. Interestingly, the same correlations exist between disease severity in growing wheat plants and mortality of actively-feeding larvae within succulent stems, and, the same isolates are responsible for greater larval mortality and plant disease severity (Wenda-Piesik et al. In preparation, Sun et al. Chapter four; In preparation). Ubiquitous *Fusarium* spp. in the soil are impacting overwintering sawfly populations in wheat fields on a yearly basis, and they are also causing larval mortality in growing wheat plants. However, there are other factors that influence saprophytic colonization of stubs by *Fusarium* spp., including tillage practices, wheat varieties, and soil moisture. These factors should be further studied, so the antagonistic relationship between *Fusarium* spp. and either the diapausing or developing larvae of wheat stem sawfly can be better understood.

Acknowledgements

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## CHAPTER FOUR

*FUSARIUM* SPP. ISOLATES CAUSE MORTALITY OF LARVAL WHEAT STEM  
SAWFLIES (HYMENOPTERA: CEPHIDAE) IN GROWING WINTER WHEAT  
PLANTS

Abstract

The pathogenicity of single isolates of three *Fusarium* spp., *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum*, obtained from colonized larval cadavers of wheat stem sawfly, was evaluated for larvae developing within growing winter wheat plants for two consecutive summer seasons in field experiments. All tested *Fusarium* isolates caused lethal effects in developing larvae inside wheat stems under natural conditions, and produced forty to eighty percent larval mortality. This reduced overall infestation in winter wheat by five to twenty percent, depending on the environmental factors. The *F. pseudograminearum* and *F. culmorum* isolates caused greater larval mortality than the *F. acuminatum* isolate. The *Fusarium* isolates also produced disease symptoms in the winter wheat plants, which included an increase in disease severity, a reduction in plant density, and yield loss. There were strong positive correlations between larval mortality and disease severity, which reflected the interchangeability of the *Fusarium* isolates between entomopathogenicity to sawfly larvae and phytopathogenicity to wheat plants. As widespread plant pathogens, *Fusarium*

spp. are also causing endemic mortality in sawfly larval populations in growing winter wheat fields annually.

### Introduction

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), was first recorded from large, hollow-stemmed, feral grasses in western United States and Canadian provinces. The insect was first considered an agricultural pest in wheat production during the early 20<sup>th</sup> century (Ainslie 1920), although Ivie (2001) hypothesizes that the insect was a recent introduction from northeastern Asia. Adoption of conservation tillage, a continuous wheat monoculture, and prolonged dry weather are primary factors contributing to the increase of sawfly populations (Morrill 1983). The direct economic losses due to sawfly infestation are a result of reduced head weight caused by larval feeding on the vascular and parenchyma tissues and by boring through nodes within the stem (Morrill and Kushnak 1996). A more apparent economic loss is due to plant lodging occurring prior to harvest. Lodged stems are difficult to harvest, and some wheat heads are not recovered. Annual losses in sawfly-infested wheat for impacted regions of North America have been reported to exceed 100 million dollars (Hartel et al. 2003, Weaver et al. 2004). These losses translate to 250 million dollars based on current wheat prices.

The wheat stem sawfly is univoltine, and its life cycle is synchronized with the physiological development of the host plants, infesting both winter and spring wheat (Morrill and Kushnak 1996). Adults emerge from obligate, overwinter diapause in the

wheat stubble which remains in the field following harvest. Adults usually appear between late May and July for areas in Montana, North Dakota, and the Canadian Prairies (Weiss et al. 1990). Male adults generally emerge first and remain near field edges where most mating occurs. However, mating is not necessary for production of viable eggs as female sawflies can control the sex of their offspring by laying either a fertilized diploid female egg or an unfertilized haploid male egg (Holmes 1982, Morrill et al. 2000). The average lifespan of the adults is 5-8 days depending on the climatic conditions and available moisture (Criddle 1923). Plants are vulnerable to attack from initiation of stem elongation through anthesis (Morrill and Kushnak 1999). A female may lay up to 50 eggs, although usually only one egg is deposited in each stem. The upper internodes of the stem are preferred. Other females may lay eggs in the same stem, but only one larva will survive due to cannibalism within the wheat stem (Wallace and McNeal 1966). The eggs take proximately 5-8 days to hatch and the average size of the newly-hatched larvae is approximately 2.2 mm in length and 0.5 mm in width (Ainslie 1920). The larvae develop within the wheat stems and feed on parenchyma and vascular tissues for a month or longer. Larval development is completed after four or five instars, depending on the host species (Farstad 1940). At maturity, the larvae can reach a length of approximately 13 mm, and a width of 2 mm. As the host plant starts to senesce, the larva moves down the stem, cuts a v-shaped notch around the stem interior, and plugs the stem below the notch with frass and plant materials (Holmes 1975). Larvae overwinter in diapause inside these plugged cut stems within the wheat residue for 8 to 9 months until pupation occurs

the following spring. Pupal development lasts from 7 to 16 days before adult emergence commences the next life cycle (Church 1955, Holmes 1975).

The life cycle of wheat stem sawfly may help to explain the difficulty in controlling populations because all immature stages occur within the protective wheat stem. The almost continuous inhabitation of stems also makes larvae more vulnerable to invasion by microorganisms colonizing both living stems and postharvest stubble. Previous field observations revealed dead and colonized sawfly larvae inside wheat stubble after harvest, and *Fusarium* spp., including *F. graminearum* Schwabe Gr1 (syn. *pseudograminearum*), *F. culmorum* (W. G. Smith) Sacc., *F. acuminatum* Ell. & Ev. sensu Gordon, *F. avenaceum* (Fr.) Sacc., and *F. equiseti* (Corda) Sacc. sensu Gordon, were isolated from the colonized larval cadavers (Wenda-Piesik et al. 2006; In preparation). These species are known to cause plant disease, such as head blight and crown or root rot in wheat in Montana (Hogg et al. 2007). However, the pathogenicity of the *Fusarium* isolates to the sawfly larvae was confirmed by laboratory bioassays and greater than 80% mortality was observed after 13 days (Wenda-Piesik et al. 2006). Subsequent greenhouse and field bioassays confirmed entomopathogenicity in spring wheat (Wenda-Piesik et al. In preparation). However, because the entomopathogenic properties of *Fusarium* spp. surviving overwinter to infect winter wheat have not been previously studied, we report on the mortality caused by the *Fusarium* isolates in cryptic wheat stem sawfly larvae within growing winter wheat plants in the field.

## Materials and Methods

### *Fusarium* Inoculum

Single isolates of three *Fusarium* spp., *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum*, were isolated from wheat stem sawfly larval cadavers that were collected in postharvest wheat fields in Pondera and Broadwater Counties, Montana, in the fall of 2001 and 2002. The isolated *Fusarium* spp. were identified by the *Fusarium* Research Center at Pennsylvania State University, and a mycological specimen of each isolate was deposited at this facility (Wenda-Piesik et al. 2006; In preparation). Single-spore cultures were rejuvenated on potato dextrose agar media at 23 °C for 7 days. Aerial mycelia were harvested by superficially scraping them from the culture surface with a scalpel and used to inoculate autoclaved oats kernels under sterile conditions. The cultures were incubated for 3 weeks at 23 °C. Colonized oat kernels were transferred to a screen-bottomed tray and air-dried in a ventilated hood for 3 days before sieving to obtain uniform-sized inoculum for experiments.

### Rearing of Adults

Sawfly-cut wheat stems containing overwintering larvae, or ‘stubs’, were collected from postharvest wheat fields near Amsterdam, Montana, during October in 2005 and 2006. The heavily-infested, susceptible hollow-stem wheat varieties were ‘McNeal’ in 2005 and ‘Reeder’ in 2006. Stubs are readily identified by the precisely cut stems and presence of the stem plugs consisting of frass. Stubs were held at 0 °C in a refrigerator for 6 months to complete larval diapause, and were then transferred to

transparent plastic boxes (40 × 20 × 20 cm) and held at 23 °C. Adults typically began emergence 6 weeks later. The males usually emerged several days earlier than females, and these newly-emerged adults were used to infest wheat plants in the field.

### Field Experiments

Field experiments were conducted at the Post Experimental Farm of Montana State University in May of 2005 and 2006. The experimental plot locations differed each year. A susceptible, hollow-stem winter wheat variety, ‘Big Sky’, was planted concurrently with inoculum at a rate of 10 grams per 3-meter row in September of 2004 and 2005 to establish treatments of *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum*. Each treatment plot was 3 × 1.2 meter and consisted of 4 rows of inoculated wheat plants, while 4 similar rows without *Fusarium* inoculum were selected as the control plot. The treatments were arranged in a randomized block design and replicated four times. Sawfly infestation was achieved using field cages at this location, which lacked an endemic population of wheat stem sawflies. A (530 μm) mesh cage (60 × 20 × 60 cm) was placed on one of the two central rows of each treatment or control plot. The wheat plants were infested during stem elongation and approximately 100 wheat plants were enclosed within each cage. To provide consistent infestation, 10 female and 5 male sawfly adults were released concomitantly into each cage. The sawfly adults were held in cages for 7 days to facilitate infestation, after which the cages were removed. The infested wheat plants were labeled and grown in the field until harvest in August. Summer weather conditions consisted of an average temperature 18.2 °C (monthly range

between 8.6 °C and 26.4 °C), average relative humidity 44.8% (monthly range between 38.6% and 54.1%) and total precipitation of approximately 18 cm.

At harvest maturity, the labeled wheat plants were collected from the plots and processed in the laboratory. In the laboratory, the number of wheat plants in each treatment was counted to record plant density and the wheat heads were subsequently separated from the stems and threshed to provide yield data. Each wheat stem was also scored on the extent of discoloration caused by the *Fusarium* isolates as 1 = none or slight, with the stems having no visible streaks or slight, darker streaks in 1 internode; 2 = moderate, with the stems having obvious purplish streaks in 2 internodes; 3 = severe, with the stems having obvious purplish streaks at 3 or more internodes. A disease rating for each *Fusarium* treatment was based upon the number of wheat stems in each classification category and the numerical value assigned to the respective category, which yields a weighted average based on the number of wheat stems showing disease symptoms and their degree of discoloration. The disease intensity rating (DR) percentage is calculated as follows:

$$\text{intensity (DR) \%} = 100(n_i D_i) / N D_{max},$$

in which  $n_i$  is the number of wheat stems of the  $i$ th category,  $D_i$  is the numerical value of the  $i$ th category,  $N$  is the total number of wheat stems in the sample, and  $D_{max}$  is the maximum category value, which is 3 (Grey and Mathre 1987).

The percentage of larval mortality for each treatment was determined by counting the number of the dead or living larvae found in the wheat stems during dissection. The percentage of stem infestation was calculated using the number of stems containing

sawfly larvae, including both larval cadavers and living larvae, from the total number of wheat stems in each treatment. Other factors infrequently causing larval mortality, such as rare attack by idiobiont parasitoids, were excluded because they only accounted for a very small proportion of larval mortality at this site. Infection by the *Fusarium* isolates was confirmed by the presence of pink, tan, or white typical fungal mycelia on the surface of sawfly larval cadavers, and confirmed by subsequent re-isolation of the *Fusarium* spp. from the larval cadavers by using single-conidia technique on both potato dextrose agar and carnation leaf agar slants for culture (Nelson et al. 1983). The larval mortality and stem infestation in the control were mainly due to naturally-occurring *Fusarium* spp., including *F. culmorum*, *F. pseudograminearum*, *F. acuminatum*, *F. avenaceum*, and *F. equiseti*.

#### Statistical Analysis

Arcsine square-root transformations were performed on all percentage data for larval mortality, disease rating, and stem infestation before analysis. A split-plot analysis of variance (ANOVA), with year as the main plot, was used to examine the effects of *Fusarium* treatments on the larval mortality, disease rating, plant density, stem infestation, and wheat yield (PROC MIXED, SAS Institute 2001). Data for both years (2006 and 2007) were combined for analysis if year showed no significant effect. Data for each year were analyzed separately by ANOVA if year showed significant effect (PROC MIXED, SAS Institute 2001). Multiple comparisons were made between the *Fusarium* treatments by separating the means using the Fisher least significance difference (LSD) at  $\alpha = 0.05$ . The relationship between larval mortality and disease rating was assessed by correlation

analysis (PROC CORR, SAS Institute 2001) and the Pearson correlation coefficient was used to assign significance at  $P < 0.05$ .

### Results

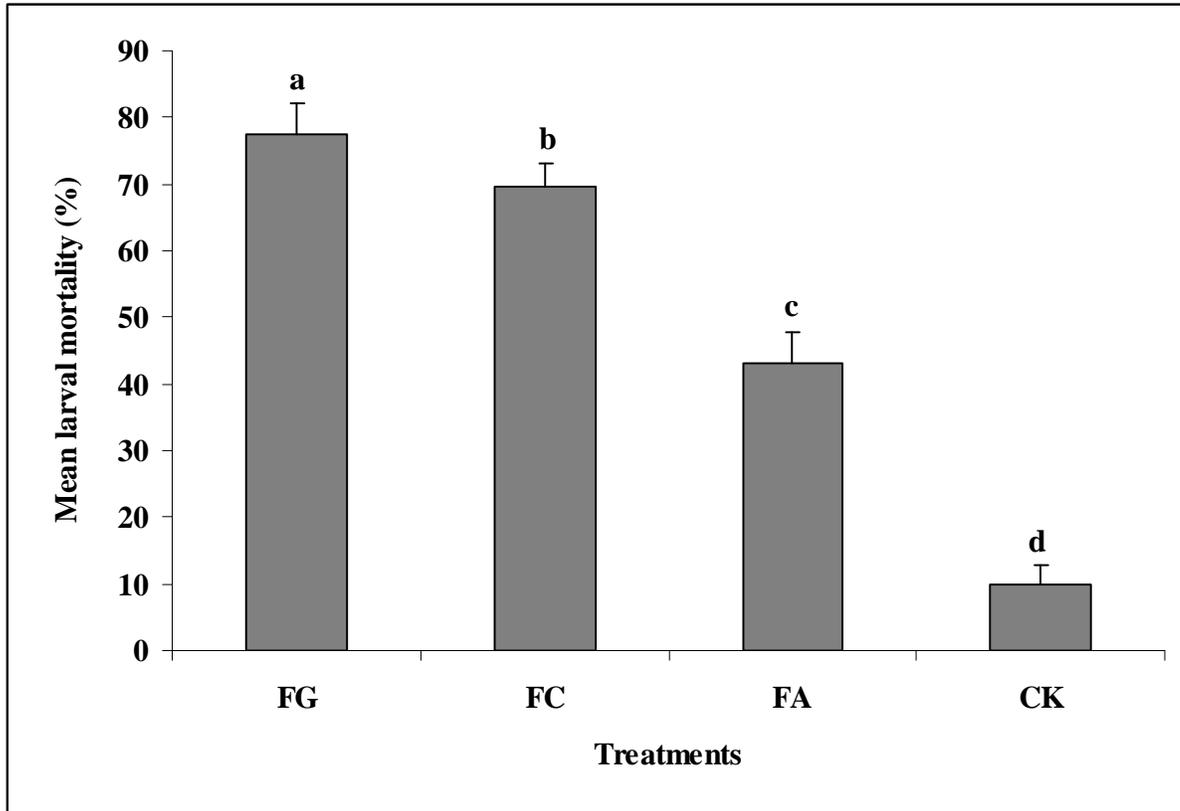


Figure 4.1. Mean mortality of larval wheat stem sawflies in *Fusarium* and control treatments in field winter wheat for both 2006 and 2007. FG - *F. pseudograminearum*, FC - *F. culmorum*, FA - *F. acuminatum*, and CK - control.

Each bar represents the combined data for treatments in both 2006 and 2007; the letters a, b, c, and d indicate significant differences between the means for the treatments; means are separated by LSD = 4.29 ( $\alpha = 0.05$ ).

In the field experiments in both 2006 and 2007, all tested *Fusarium* isolates showed significant pathogenicity to the larvae developing inside the wheat stems during the growing season ( $F = 370.14$ ,  $df = 3$ ,  $P < 0.001$ ) (Figure 4.1). The mean larval

mortality did not differ significantly between years ( $F = 0.47$ ,  $df = 1$ ,  $P = 0.541$ ), and the interaction between year and treatment was not significant ( $F = 1.47$ ,  $df = 3$ ,  $P = 0.256$ ). At the time of the harvest sampling, the mean larval mortality in the *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* treatments was 77.5%, 69.5%, and 43.0% respectively. The mean larval mortality in the control was 9.9%, which was significantly lower than for the *Fusarium* treatments. The *F. pseudograminearum* isolate caused the greatest mortality in the larvae, while the *F. culmorum* isolate caused significantly greater mortality in the larvae than the *F. acuminatum* isolate (Figure 4.1).

The disease rating of wheat plants in the *Fusarium* treatments reflected the phytopathogenicity of the colonizing isolates. The mean disease rating did not differ significantly between years ( $F = 2.80$ ,  $df = 1$ ,  $P = 0.193$ ), and the interaction between year and treatment was not significant ( $F = 1.51$ ,  $df = 3$ ,  $P = 0.247$ ). When sampled at harvest, the mean disease rating for the *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* treatments was 70.9%, 67.6%, and 54.2% respectively. This was significantly greater than the mean disease rating for the control, which was 42.8% ( $F = 233.63$ ,  $df = 3$ ,  $P < 0.001$ ) (Figure 4.2). The *F. pseudograminearum* and *F. culmorum* isolates caused significantly greater disease ratings in wheat stems than the *F. acuminatum* isolate, while the *F. pseudograminearum* isolate caused a significantly greater disease rating than the *F. culmorum* isolate (Figure 4.2). The *Fusarium* isolates caused pathogenicity in both wheat plants and sawfly larvae during the growing season and there was a significant positive correlation between wheat disease rating and sawfly

larval mortality across the *Fusarium* and control treatments in the field experiments ( $n = 32$ ,  $r = 0.957$ ,  $P < 0.001$ ) (Figure 4.3).

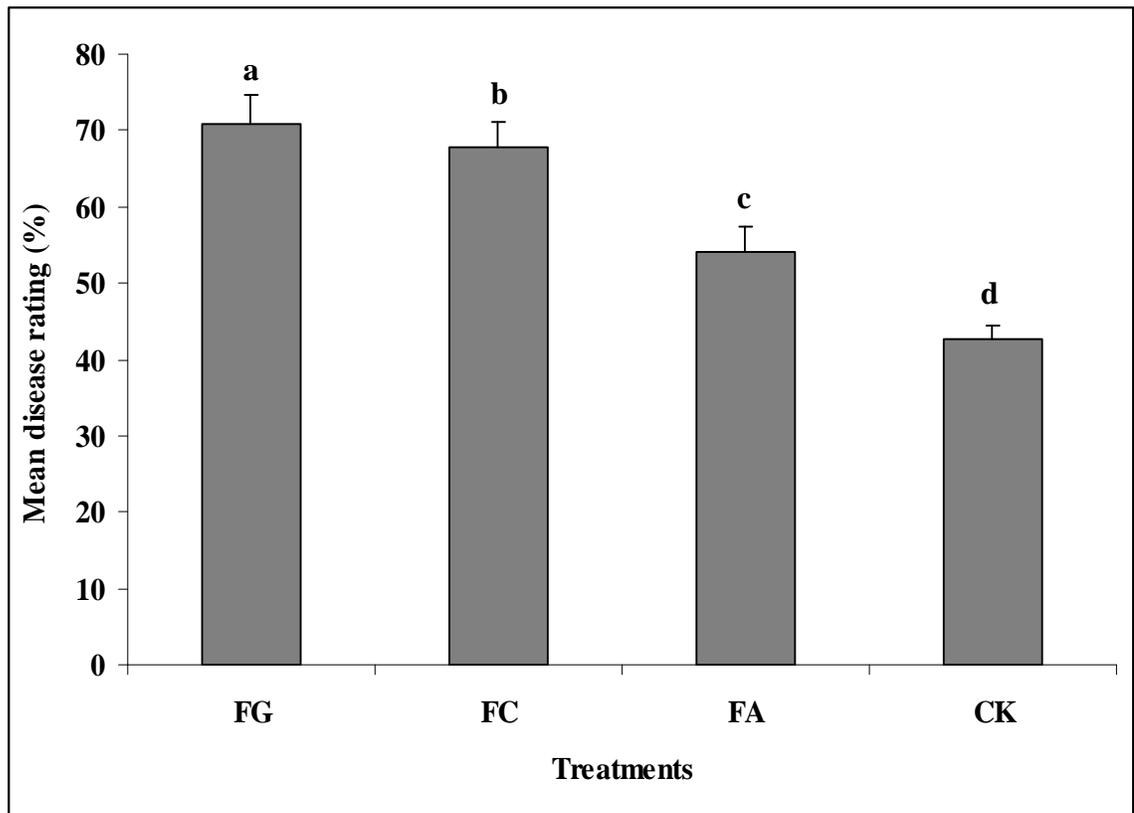


Figure 4.2. Mean disease ratings for wheat plants in *Fusarium* and control treatments in field winter wheat for both 2006 and 2007. FG - *F. pseudograminearum*, FC - *F. culmorum*, FA - *F. acuminatum*, and CK - control.

Each bar represents the combined data for both 2006 and 2007; the letters a, b, c, and d indicate significant differences between the means for the treatments; means are separated by  $LSD = 2.91$  ( $\alpha = 0.05$ ).

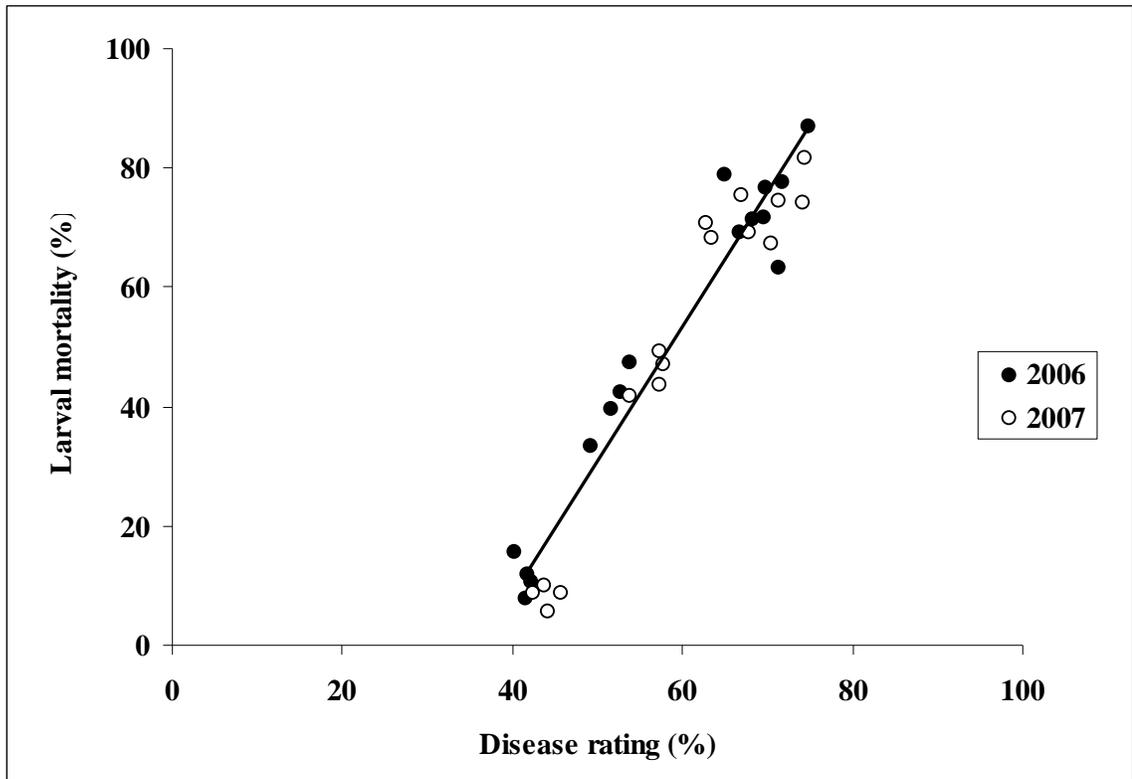


Figure 4.3. Correlation between mortality of larval wheat stem sawflies and wheat disease rating in field experiments in 2006 and 2007.

Table 4.1. Plant density, stem infestation, and wheat yield of *Fusarium* treated and control winter wheat plants in field experiments in 2006 and 2007.

Treatment	Plant density (stems m <sup>-1</sup> )		Stem infestation (%)		Wheat yield (g m <sup>-1</sup> )
	(Mean ± SE)		(Mean ± SE)		(Mean ± SE)
	2006	2007	2006	2007	2006 - 2007
<i>F. pseudograminearum</i>	141.9 ± 9.3 c	156.2 ± 11.5 b	33.4 ± 5.6 b	52.3 ± 2.2 a	117.0 ± 11.3 c
<i>F. culmorum</i>	168.1 ± 12.4 b	199.7 ± 17.4 a	36.6 ± 6.9 b	47.6 ± 3.7 a	135.2 ± 12.6 b
<i>F. acuminatum</i>	178.4 ± 20.1 b	227.6 ± 34.3 a	51.9 ± 4.6 a	46.9 ± 6.8 a	144.7 ± 11.3 b
Control	200.1 ± 14.7 a	228.0 ± 4.2 a	53.4 ± 5.4 a	46.2 ± 4.8 a	163.9 ± 22.9 a

The wheat yield data were pooled for the treatments of both 2006 and 2007; within a column, means followed by the same letter are not significantly different (LSD test;  $\alpha = 0.05$ );

The *Fusarium* isolates also caused pathogenic effects in plant density in 2006 ( $F = 17.53$ ,  $df = 3$ ,  $P = 0.0004$ ) and in 2007 ( $F = 11.21$ ,  $df = 3$ ,  $P = 0.0021$ ) (Table 4.1). The mean plant density varied among years ( $F = 27.36$ ,  $df = 1$ ,  $P = 0.013$ ). However, the

interaction between year and treatment was not significant ( $F = 1.49$ ,  $df = 3$ ,  $P = 0.251$ ). The plant density in the *Fusarium* treatments was higher in 2007 than in 2006. The *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* isolates reduced mean plant density by 29.1%, 16.0%, and 10.9%, respectively in 2006, and 31.5%, 12.4%, and 0.2%, respectively in 2007, compared to the control. Theoretically, the reduced plant density in *Fusarium* treatments would result in higher stem infestation by sawflies as the wheat plants in each treatment were facing the same sawfly pressure in the cage. However, the mean percentage of stem infestation in the *F. pseudograminearum* and *F. culmorum* treatments was significantly lower than that in the control for 2006 ( $F = 13.18$ ,  $df = 3$ ,  $P = 0.0012$ ) (Table 4.1). There was no significant difference in mean percentage of stem infestation between treatments in 2007 ( $F = 1.40$ ,  $df = 3$ ,  $P = 0.305$ ), but overall percentage of stem infestation in the *F. pseudograminearum* and *F. culmorum* treatments was still quite low considering the lower overall plant density between treatments. The mean stem infestation did not differ between years ( $F = 5.95$ ,  $df = 1$ ,  $P = 0.0926$ ), but the interaction between year and treatment was significant ( $F = 11.67$ ,  $df = 3$ ,  $P = 0.0002$ ). Between 2006 and 2007, there was an obvious increase in the mean stem infestation in the *F. pseudograminearum* and *F. culmorum* treatments, but not in the *F. acuminatum* and control treatments, which explains the interaction (Table 4.1).

The negative impact of the *Fusarium* isolates on wheat yield was dramatic, and significant differences in wheat yield were evident among treatments ( $F = 35.74$ ,  $df = 3$ ,  $P < 0.001$ ) (Table 4.2). The mean wheat yield did not differ significantly between years ( $F = 1.43$ ,  $df = 1$ ,  $P = 0.317$ ), and the interaction between year and treatment was not

significant ( $F = 2.64$ ,  $df = 3$ ,  $P = 0.081$ ). The *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* isolates caused 28.6%, 17.5%, and 11.7% yield reductions respectively, when compared to the control (Table 4.1). The yield losses in the *Fusarium* treatments were primarily due to the reduced plant density and the pathogenicity of the *Fusarium* isolates to the wheat plants rather than sawfly infestation, because the *Fusarium* isolates could reduce yield losses due to sawflies by killing young larvae and reducing the stem infestation in the growing winter wheat plants.

### Discussion

In these experiments, we found that the tested *Fusarium* isolates were lethal to sawfly larvae, and they also caused disease symptoms in winter wheat plants during the growing season. Therefore, these *Fusarium* isolates could colonize wheat tissues as plant pathogens, and could also attack wheat stem sawfly larvae as insect pathogens. The entomopathogenic and phytopathogenic properties of those *Fusarium* isolates have been studied in spring wheat as well, and high larval mortality and disease severity were recorded in the field experiments (Wenda-Piesik et al. In preparation). Stem colonization of winter wheat by *Fusarium* can be directly related to the incidence of infection of seeds at planting in the fall, and of seedlings in the next spring (Duthie and Hall 1987, Clement and Parry 1998). Winters in the state of Montana are characterized by fluctuating temperatures with low humidity and precipitation. The *Fusarium* spp. are known to produce macroconidia and chlamydospores that help them to survive and remain biologically active over an unusually wide range of temperature and moisture levels for

years (Wilcock and Megan 2001). The data for larval mortality and disease ratings in this study support that these *Fusarium* isolates could survive overwinter to infect winter wheat and sawfly larvae the next growing season.

As plant pathology and entomology have been traditionally pursued as independent disciplines, the dual role of *Fusarium* spp. acting as both plant pathogens and insect pathogens has not been widely studied. Several *Fusarium* spp. were frequently isolated from growing wheat plants as plant pathogens, including *F. culmorum*, *F. pseudograminearum*, *F. acuminatum*, *F. avenaceum*, and *F. equiseti* (Klaasen et al. 1991, Luque et al. 2005). Phytopathogenic *Fusarium* spp. also occasionally cause epizootics in insect populations in the field (Strongman et al. 1988, Dowd et al. 1989). In fact, entomopathogenic fungi could have evolved from phytopathogenic fungi via adaptations in extracellular hydrolytic enzymes so as to accommodate hydrolysis of proteinaceous insect cuticles. Some plant pathogens possess structural and behavioral adaptations which are very similar to entomopathogens, and the underlying mechanisms of fungal pathogenesis maybe similar in insects and plants (St Leger et al. 1997). Many *Fusarium* spp. are known to produce a broad spectrum of enzymes for protein and polysaccharide hydrolysis, which could be useful in completing the breakdown of complex organic substances, including both living and non-living plant cell walls and insect cuticles. In addition, *Fusarium* spp. could produce mycotoxins which may be active against both plants and insects (Diener et al. 1987, Abbas and Mulrooney 1994). For example, *F. graminearum* Schwabe is a pathogen of wheat, corn, and other cereals in northern temperate climates and it could produce mycotoxins, including trichothecene

deoxynivalenol, zearalenone, and approximately 40 other secondary metabolites, all of which could be pathogenic to both plants and insects (Dowd et al. 1989). Deoxynivalenol was also detected in wheat stem tissues colonized by the *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* isolates in two years of field experiments. The deoxynivalenol caused toxicity and inhibited growth of developing larvae in diet bioassays when administered at the naturally-occurring concentrations for wheat stems colonized by *Fusarium* spp. (Sun et al. Chapter five; In preparation).

The versatility of *Fusarium* spp. in transforming from plant pathogens to insect pathogens appears to facilitate the attack of both wheat plants and sawfly larvae in the field, which could explain the strong positive correlation between disease rating and larval mortality in the *Fusarium* treatments. In this study, the *Fusarium* isolates showed varying aggressiveness in pathogenicity to both wheat plants and sawfly larvae in the two years of field experiments. The *F. pseudograminearum* and *F. culmorum* isolates caused significantly greater disease in winter wheat and also caused significantly greater larval mortality than the *F. acuminatum* isolate. The decreased larval mortality could be attributed to the reported weakness of *F. acuminatum* as a plant pathogen when compared to *F. pseudograminearum* and *F. culmorum* (Xue et al. 2004). *Fusarium* spp. are also associated with decreased emergence of seedlings and lower stand densities (Kane and Smiley 1987), and plant density was reduced by ten to thirty percent in our *Fusarium* treated plots, which probably accounted for the major yield losses observed in this study. When considering the plant density, the lower stem infestation in the *Fusarium* treatments supported the fact that the *Fusarium* isolates could impose strong negative

impacts on sawfly larvae, and reduce the overall stem infestation during the growing season.

In summary, all tested *Fusarium* isolates had lethal impacts on the sawfly larvae feeding inside winter wheat stems under natural conditions during the growing season. They could account for a forty to eighty percent decrease in the annual larval sawfly population, and could reduce stem infestation by five to twenty percent, depending on the environmental factors in the field. The *F. pseudograminearum* and *F. culmorum* isolates caused greater larval mortality than the *F. acuminatum* isolate. However, the tested *Fusarium* isolates also caused a ten to thirty percent increase in the severity of wheat disease and a ten to thirty percent reduction in plant density, which probably accounted for the ten to thirty percent decrease in wheat yield. The strong correlation between larval mortality and disease rating for infected winter wheat in the *Fusarium* treatments reflects the interchangeability of the *Fusarium* isolates between entomopathogenicity to sawfly larvae and phytopathogenicity to wheat plants. Although they are ubiquitous plant pathogens, *Fusarium* spp. are impacting sawfly larval populations in the wheat fields annually. Factors that could influence the pathogenicity of *Fusarium* spp. to sawfly larvae, including wheat variety, winter or spring wheat, soil moisture, and fungicide applications should be further studied. The antagonistic relationship between *Fusarium* spp. and sawfly larvae during wheat growing season must be carefully considered when developing integrated pest management strategies for wheat stem sawfly.

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## CHAPTER FIVE

TOXICITY AND SUBLETHAL EFFECTS OF DEOXYNIVALENOL TO LARVAE  
OF THE WHEAT STEM SAWFLY, *Cephus cinctus* NORTONAbstract

Deoxynivalenol, commonly called 'DON', was detected in wheat stem tissues that had been colonized by *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* in field experiments conducted for each of two years. DON caused toxicity and growth inhibition of actively-feeding larvae in diet bioassays when administered at levels occurring in wheat stems colonized by *Fusarium*. Developing larvae in different growth stages varied in their sensitivity to DON, and the larger larvae had greater tolerance of DON. Toxicity also occurred when DON was injected into larvae in diapause. This study indicates that, DON, the most commonly detected trichothecene mycotoxin in wheat, is a mortality factor for larval populations of the wheat stem sawfly in plants infected by *Fusarium* spp. in wheat fields.

Introduction

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is a widely distributed and destructive wheat insect pest in Montana, North Dakota, South Dakota, Nebraska, Colorado, Wyoming, Alberta, Saskatchewan, and Manitoba (Weiss and Morrill 1992). Annual losses in all production areas were reported to exceed 100

million dollars (Hartel et al. 2003, Weaver et al. 2004), which is at least 250 million dollars at current prices. The wheat stem sawfly is univoltine on wheat and most other grasses that occur in this area (Morrill and Kushnak 1996). Sawfly adults emerge from obligate diapause between late May and July for areas in Montana, North Dakota, and Canada (Weiss et al. 1990). Females oviposit in the stem lumen, and all immature stages, including either four or five actively-feeding larval instars, remain within the wheat stems (Farstad 1940, Wallace and McNeal 1966). Feeding on parenchyma and vascular tissues lasts for a month or longer. Last-instar larvae descend within the mature, desiccated stems, cut an internal v-shaped notch at the stem base, and plug the stem below the notch with frass and plant materials just before harvest. The larvae overwinter in diapause inside a hibernaculum within the cut stem for 8 to 9 months until pupation occurs the next spring (Church 1955, Holmes 1975).

The biology of wheat stem sawfly can explain much of its pest status, because losses are due to feeding and stem cutting within the protection of wheat stems. However, location in the stem interior also makes the larvae more susceptible to microorganisms colonizing wheat stems, as well as to mycotoxins produced by these microorganisms. Previous research has reported dead and colonized larvae in wheat stems (Wenda-Piesik et al. In preparation, Sun et al. Chapter three; In preparation). Single *Fusarium* isolates, belonging to the species of *F. graminearum* Schwabe Gr1 (syn. *pseudograminearum*), *F. culmorum* (W. G. Smith) Sacc., *F. acuminatum* Ell. & Ev. sensu Gordon, *F. avenaceum* (Fr.) Sacc., and *F. equiseti* (Corda) Sacc. sensu Gordon, were isolated from the colonized sawfly larval cadavers (Wenda-Piesik et al. 2006; In preparation, Sun et al. Chapter one;

In preparation). Several of these *Fusarium* spp. are generally known to cause plant diseases such as *Fusarium* head blight, and *Fusarium* crown or root rot in wheat grown in Montana (Hogg et al. 2007). However, the pathogenicity of these *Fusarium* species to growing sawfly larvae was confirmed through field experiments by inoculating winter wheat plants with *Fusarium* isolates and subsequently infesting wheat plants with sawfly adults. Mean larval mortality of 77.5%, 69.5%, and 43.0% was observed in wheat stems infected by *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* respectively, over two years in field experiments (Sun et al. Chapter four; In preparation). In addition, *Fusarium* spp. produce mycotoxins with activity against both plants and insects (Diener et al. 1987, Abbas and Mulrooney 1994). For example, *F. graminearum* Schwabe is a pathogen of wheat, corn and other cereals in northern temperate climates where it produces mycotoxins, including the trichothecenes deoxynivalenol (DON) and zearalenone, plus approximately 40 other secondary metabolites (Dowd et al. 1989), all of which could be pathogenic to both plants and insects. The presence of *Fusarium* toxins in wheat stem tissues could increase the pathogenicity of *Fusarium* isolates to sawfly larvae. The goal of this research was to measure toxin amounts in wheat stem tissue infected by *Fusarium*, and to establish the lethal dosage relationships for DON and sawfly larvae in laboratory bioassays. The thresholds were compared to naturally-occurring DON levels in wheat stems infected by several *Fusarium* species.

## Materials and Methods

### Quantification of *Fusarium* Toxins

Field experiments were conducted at the Post Experimental Farm of Montana State University. A susceptible, hollow-stem winter wheat variety, 'Big Sky', was planted concurrently with inoculum (*Fusarium* colonized oat kernels) at a rate of 10 grams per 3-meter row in September of 2004 and 2005 to establish treatments of *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum*. Each treatment plot was 3 × 1.2 meter and comprised of 4 rows of inoculated wheat plants (Sun et al. Chapter four; In preparation). There was a single sampling event at harvest in August of 2006, and there were three sampling events during the growing season in June, July, and August of 2007. Stems were randomly collected from the wheat plants inoculated with *Fusarium*. The collected wheat stems were air-dried and ground into powder using a 1 mm screen in a laboratory mill machine. The stem tissues were quantitatively analyzed for toxins using gas chromatography-mass spectroscopy at the Veterinary Diagnostic Laboratory of North Dakota State University (Raymond et al. 2003).

### Collection of Sawfly Larvae

Growing wheat plants containing larval sawflies were collected from the winter wheat fields near Choteau and Conrad, Montana in July of 2007. The two locations are approximately 100 km apart, and the wheat variety was 'Neeley' and 'NuHorizon' in Choteau and Conrad respectively. The wheat stems were individually dissected in the laboratory to obtain developing larvae. The 2<sup>nd</sup>-instar larvae ranged between 3.0 to 4.0

mg in pretreatment weight, while the 3<sup>rd</sup>-instar larvae ranged between 4.0 to 5.0 mg in pretreatment weight. Larvae were held separately for the toxin bioassay.

Sawfly-cut wheat stems containing larvae in diapause were collected from postharvest spring wheat fields near Amsterdam, Montana, in October of 2006. The sawfly-cut stems containing larvae were kept in the refrigerator at 0 °C for 2 months before they were individually dissected in the laboratory to obtain diapausing larvae for the toxin bioassay.

#### Diet Bioassay

Commercial diet for fall armyworm *Spodoptera frugiperda* (J. E. Smith) (F9179B, BioServ, Frenchtown, New Jersey) was autoclaved for 15 min before mixing with DON (Sigma, D-0156) aqueous solutions prepared with double distilled water, and were dispensed into transparent plastic sterile centrifuge tubes (2 ml) containing about 1 ml diet each (Macedo et al. 2005). The final concentrations of DON in the diet were 40, 10, and 2.5 µg/ml, while diet without DON served as the control. The diet in the centrifuge tube was punctured with a sterilized glass stirring rod to create a well (3 mm in diameter and 10 mm in length) on the edge, which simulated wheat stem structure and enabled subsequent observation of the larval growth and mortality on the diet. Growing larvae were individually weighed before being introduced singly into each diet well with a camel hair brush. The centrifuge tubes with larvae were sealed using parafilm with a tiny ventilation hole (0.5 mm in diameter), and kept at 23 °C under complete darkness. There were 8 larvae in each treatment with 3 replications. The larval mortality was inspected every 2 days, and the surviving larvae were weighed individually after the 10

day exposure. Larval growth inhibition was assayed relative to control based on larval weight gained through 10 days of feeding on the treated diet. The growth inhibition was calculated from this equation: growth inhibition =  $[(C_L - T_L)/C_L] \times 100$ ; where  $C_L$  is the larval weight gained in the control and  $T_L$  is the larval weight gained in the treatment (EI-Aswad et al. 2003). Since larval mortality levels were initially very low across all the treatments, only the values from the examination at 10 days were reported and analyzed.

### Injection Bioassay

DON was diluted using double distilled water to yield concentrations of  $10^4$ ,  $10^3$ ,  $10^2$ , 10, and 1  $\mu\text{g/ml}$ . Diapausing sawfly larvae received DON by injection of 1  $\mu\text{l}$  solution into the larval hemolymph using a 10- $\mu\text{l}$  syringe with an 18-gauge needle. The diapausing larvae in the control were injected with water only. There were 10 larvae in each treatment with 3 replications. The treated sawfly larvae were held in Petri dishes lined with filter paper at 23 °C, and larval mortality was recorded on each day in the 6 day experimental period. Larval mortality was very low for the first three days across the treatments, and the larval mortality was more than 30% in the control treatment on the 6<sup>th</sup> day post treatment, so only larval mortality data on the 4<sup>th</sup> and 5<sup>th</sup> day were reported and analyzed.

### Statistical Analysis

The data on toxin levels in wheat stem tissues colonized by *Fusarium* were not analyzed because there were no replications for each fungal species within a sampling event. The mortality of diapausing larvae caused by DON in the injection bioassay was

analyzed by probit analysis (PROC PROBIT, SAS Institute 2001). Arcsine square-root transformations were performed on all percentage data for growth inhibition and larval mortality of developing larvae before analyzed using analysis of variance (ANOVA) (PROC MIXED, SAS Institute 2001). Multiple comparisons were made between the DON treatments by separating the means using the Fisher least significance difference (LSD) at  $\alpha = 0.05$ .

## Results

### Quantification of *Fusarium* Toxins

*Fusarium* toxins, including deoxynivalenol, or DON, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, and nivalenol were detected in the stem tissues, but the levels of the latter 3 toxins were lower than 0.5  $\mu\text{g/ml}$  in both 2006 and 2007. When the plant samples were collected in August of 2006, the levels of DON in stem tissues that were colonized by the single isolates of *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* were 30.5, 13.9, and 5.2  $\mu\text{g/ml}$ , respectively (Table 5.1). For the samples that were collected from June through August of 2007, the levels of DON for *F. pseudograminearum* increased from 3.5 to 13.8  $\mu\text{g/ml}$ , while the levels of DON for *F. culmorum* and *F. acuminatum* were 3.5 and 1.5  $\mu\text{g/ml}$  respectively, at the end of the season.

### Diet Bioassay

The greatest mortality of 2<sup>nd</sup>-instar larvae collected from growing wheat stems resulted from consumption of diet containing 40  $\mu\text{g/ml}$  of DON, while there was no

significant difference in mortality between the other treatments and the control (Table 5.2). Growth of 2<sup>nd</sup>-instar larvae was significantly inhibited when the DON concentration in the diet was greater than 10 µg/ml, while DON caused no significant effects on the growth of 2<sup>nd</sup>-instar larvae at concentrations less than 2.5 µg/ml. The 3<sup>rd</sup>-instar larvae were less susceptible to DON (Table 5.3). Larval mortality was very low across all treatments, and there was no significant difference between the treatments and the control. Growth of 3<sup>rd</sup>-instar larvae was also significantly inhibited by DON when administrated at 10 and 40 µg/ml in diet, while DON showed no effect on growth of these larger larvae at 2.5 µg/ml or less.

Table 5.1. The amount of DON (µg/ml) in wheat stem tissues colonized by *Fusarium* isolates.

<i>Fusarium</i> species	Year			
	2006	2007		
	August	June	July	August
<i>F. pseudograminearum</i>	30.5	3.5	3.5	13.8
<i>F. culmorum</i>	13.9	1.0	< 0.5	3.5
<i>F. acuminatum</i>	5.2	< 0.5	< 0.5	1.5

Table 5.2. Relative toxicity of DON to 2<sup>nd</sup>-instar larvae after 10 days in a diet bioassay.

Concentration (µg/ml)	Pre-treat weight (mg) (Mean ± SE)	Post-treat weight (mg) (Mean ± SE)	Growth inhibition (%)	Mortality (%)
40	3.4 ± 0.3 a	5.7 ± 0.7 d	68.2 a	12.5 a
10	3.6 ± 0.3 a	8.3 ± 1.0 c	32.5 b	4.2 b
2.5	3.5 ± 0.3 a	10.0 ± 1.8 b	6.8 c	0 b
0	3.6 ± 0.4 a	10.6 ± 1.9 a	0 c	0 b

In each column means followed by the same letter are not significantly different by LSD ( $\alpha = 0.05$ ).

Table 5.3. Relative toxicity of DON to 3<sup>rd</sup>-instar larvae after 10 days in a diet bioassay.

Concentration ( $\mu\text{g/ml}$ )	Pre-treat weight (mg) (Mean $\pm$ SE)	Post-treat weight (mg) (Mean $\pm$ SE)	Growth inhibition (%)	Mortality (%)
40	4.4 $\pm$ 0.3 a	10.2 $\pm$ 0.9 c	46.3 a	4.2 a
10	4.5 $\pm$ 0.4 a	12.9 $\pm$ 1.5 b	21.1 b	0 a
2.5	4.4 $\pm$ 0.3 a	14.9 $\pm$ 1.9 a	1.9 c	0 a
0	4.4 $\pm$ 0.3 a	15.2 $\pm$ 1.9 a	0 c	0 a

In each column means followed by the same letter are not significantly different by LSD ( $\alpha = 0.05$ ).

### Injection Bioassay

In the experiment using larvae in diapause, solutions of DON caused considerable mortality when delivered by injection (Table 5.4). The lethal dosage required to kill 50% of test population was 5.90  $\mu\text{g}$  and 1.56  $\mu\text{g}$  on the 4<sup>th</sup> and 5<sup>th</sup> day after treatment, respectively. The range of LD<sub>50</sub> values represents a near four-fold difference in larval susceptibility to DON with incubation for an additional day. The lethal dosage to kill 90% larval population reduced from 1501  $\mu\text{g}$  to 378.85  $\mu\text{g}$  between the 4<sup>th</sup> day and 5<sup>th</sup> day after treatment, respectively (Table 5.4). The range of LD<sub>90</sub> values remains 4 times greater after an additional day of incubation due to the similarity in the slope for each day.

Table 5.4. Lethal dosage ( $\mu\text{g}$ ) of DON for diapausing sawfly larvae in an injection bioassay, based on probit analysis of mortality after incubation for 4 and 5 days.

Day	Slope (SE)	Intercept (SE)	LD <sub>50</sub> (95% FL)	LD <sub>90</sub> (95% FL)	$\chi^2$ (P)
4	0.91 (0.20)	-0.70 (0.26)	5.90 (1.61-46.53)	1501.00 (132.51-403949.00)	21.56 (< 0.001)
5	0.92 (0.33)	-0.18 (0.35)	1.56 (0.06-6.66)	378.85 (43.00-1902.89)	7.90 (0.005)

### Discussion

The presence of DON in wheat stem tissues colonized by *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* was confirmed in this study during vegetative growth and at harvest. Protein synthesis in eukaryotic cells could be inhibited by DON, thus suppressing host immunity to fungal colonization. Production of DON results in an aggressive invasion of fungi through wheat heads, causing *Fusarium* head blight (Bai et al. 2001). Isolates of *F. pseudograminearum* have been reported to extensively colonize wheat plants, including heads, stems, and crowns, by producing a significant amount of DON, and DON levels could reach 111 µg/ml in *Fusarium*-infected crowns (Mudge et al. 2006). In this study, the late season levels of DON in wheat stem tissues colonized by *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* were greater than 5 µg/ml in 2006, and DON was also present in wheat stems colonized by the same species at three sampling events during the summer of 2007. Thus, DON can play an important role in facilitating the colonization of wheat stems and the subsequent attack of sawfly larvae by *Fusarium* species.

In the diet bioassays, DON caused toxicity and inhibited the growth of the developing sawfly larvae at the concentrations used in this study. The amount of DON in *Fusarium*-infected wheat stem tissues ranged between 1.5 and 30.5 µg/ml in the field trials over two years and the DON concentrations used in the diet bioassay simulated the toxin levels occurring under field conditions. This provides valuable data about the debilitating or lethal effects of DON on sawfly larvae in wheat stems under field

conditions. Combinations of DON with other *Fusarium* metabolites have been reported to cause synergistic mortality in *S. frugiperda* (J. E. Smith) (Dowd et al. 1989). Other secondary metabolites produced by *Fusarium* spp. in this study, including 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, and nivalenol, may be also involved in toxicity to developing sawfly larvae via synergism, even when present only at trace levels in wheat stem tissues.

Sawfly larvae varied in their sensitivity to DON as a function of size, and the more developed larvae had greater tolerance of DON. For example, when DON was administered at 40 µg/ml in the diet, growth inhibition and larval mortality were 46.3% and 4.2%, respectively for 3<sup>rd</sup>-instar larvae, while growth inhibition and larval mortality were 68.2% and 12.5%, respectively for 2<sup>nd</sup>-instar larvae. The pretreatment larval weights were 0.8-1.0 mg larger for the later instar (Table 5.2 and Table 5.3). The 1<sup>st</sup>-instar larvae did not survive well on the diet. This prevented the determination of any lethal effects of DON on neonates or small larvae, which theoretically should be even more sensitive to DON. So, the actual impact of DON on developing larvae can be more dramatic in the field if the newly-hatched larvae consumed stem tissues containing DON.

DON also caused mortality in diapausing sawfly larvae in the injection bioassay. Injection of DON into the larval haemolymph could compromise immunity, as a result of immunosuppression, including inhibiting protein synthesis, and causing cytotoxic radiomimetic-like lesions in the rapidly dividing cells of the hematopoietic and lymphoid tissue, as was found for numerous laboratory animals (Corrier 1991). The toxicity of DON was also reported in Lepidopteran (*Spodoptera frugiperda*) cell lines (Fornelli et al.

2004). The immune function of insects partially depends on fat body reserves (Schmid-Hempel 2005), which explains the higher tolerance of DON in the larger sawfly larvae. The larger fat reserves in diapausing larvae might also decrease their sensitivity to DON. The toxic potential of DON to diapausing larvae could be achieved through the initial penetration of *Fusarium* fungal mycelia and subsequent production of DON in vivo.

In summary, DON was detected in wheat stems colonized by *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* in field experiments for two years. This toxin caused mortality and inhibited the growth of actively-feeding larvae in diet bioassays when administered at naturally-occurring concentrations from *Fusarium*-colonized wheat stems. Developing larvae in different stages varied in their sensitivity to DON and the larger larvae were more tolerant to DON. The injection bioassay indicated that DON also caused lethal effects in diapausing larvae, even though larval physiology is attenuated in this phase. These results suggest that DON, as the most commonly detected trichothecene mycotoxin of wheat, can be a mortality factor in larval populations of wheat stem sawfly in wheat fields.

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Progress in Plant Protection 46: 380-387.

## CHAPTER SIX

MORTALITY FACTORS OF WHEAT STEM SAWFLY, *Cephus cinctus* NORTON,  
LARVAE IN DRYLAND AND IRRIGATED WHEAT FIELDSAbstract

Wheat stem sawfly infestation and larval survival in both dryland and irrigated wheat fields was studied at multiple locations in Montana over 3 years. Overall, in both field experiments and field surveys, there were no obvious differences in either sawfly infestation or larval survival in dryland and irrigated wheat fields. Attack by braconid parasitoids was the major lethal factor in developing larvae during the summer, while fungal infection, primarily by *Fusarium* spp., caused significant mortality of larvae in diapause overwinter. Anecdotal grower observations of greater sawfly infestation in dryland fields can be due to different annual cropping and cultivation practices for dryland and irrigated wheat fields.

Introduction

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is a widely distributed and destructive insect pest in wheat production in the northern Great Plains, including Montana, North Dakota, South Dakota, Nebraska, Colorado, Wyoming, Alberta, Saskatchewan, and Manitoba (Weiss and Morrill 1992). This species was first considered an agricultural pest in wheat production in the early 20<sup>th</sup> century (Ainslie

1920). The adoption of conservation tillage, the long-standing monoculture of wheat, and frequent, prolonged dry weather conditions are the primary factors contributing to the increase of sawfly populations (Weaver et al. 2004). The direct economic losses due to infestation are a reduction in kernel weight and grain quality as larval feeding on the vascular and parenchyma tissues of wheat stems (Morrill and Kushnak 1996). However, the most visible loss is the lodging of wheat stems when the mature larva cuts an internal v-shaped notch at the stem base of the desiccated plant at the time of harvest. Recovery of lodged stems is very difficult, and annual losses over all impacted wheat production regions have been reported to exceed 100 million dollars (Hartel et al. 2003, Weaver et al. 2004), which is at least 250 million dollars in the current market.

The wheat stem sawfly completes one life cycle per year on wheat or grass hosts, and its life cycle is synchronized with the physiological development of the host plants (Morrill and Kushnak 1996). Adults emerge from obligate diapause in wheat stubble from the previous year between late May and July for areas in Montana, North Dakota, and Canada (Criddle 1923, Weiss et al. 1990). The developmental stages susceptible to sawfly oviposition begin with stem elongation (stage 31) through anthesis (stage 69) on the Zadoks scale (Zadoks et al. 1974). The females tend to deposit eggs in the uppermost internodes of suitable stems. Egg hatch requires 5-8 days in wheat stems, and feeding larvae forage along the length of the stem. Ultimately, only one larva completes its life cycle per stem, and all other immatures are cannibalized (Wallace and McNeal 1966). The developing larvae within the wheat stems feed on parenchyma and vascular tissues for a month or longer, and pass through four or five instars, depending on conditions

(Farstad 1940). As the host plant starts to senesce, the surviving larva moves to the bottom of the stem, cuts a notch around the stem lumen, and plugs the interior space with frass and plant materials (Holmes 1975). The larvae overwinter in diapause within hibernacula inside sawfly-cut stems for 8 to 9 months until pupation occurs the next spring. Metamorphosis typically requires 7 to 16 days, and the adults emerge to start a new life cycle when conditions are favorable (Church 1955, Holmes 1975). There are natural enemies that impact wheat stem sawfly populations. Parasitoids, mainly *Bracon cephi* (Gahan) and *Bracon lissogaster* Muesebeck, attack developing larvae inside wheat stems. Both *B. cephi* and *B. lissogaster* have two generations annually, and the levels of parasitism ranged from 15% to 98% in fields around Montana (Morrill et al. 1994, Morrill et al. 1998). Several *Fusarium* spp., including *F. graminearum* Schwabe Gr1 (syn. *pseudograminearum*), *F. culmorum* (W. G. Smith) Sacc., *F. acuminatum* Ell. & Ev. sensu Gordon, *F. avenaceum* (Fr.) Sacc., and *F. equiseti* (Corda) Sacc. sensu Gordon, were found to be pathogenic to both actively-feeding larvae in growing wheat plants, and to diapausing larvae in sawfly-cut stems, called ‘stubs’ (Wenda-Piesik et al. 2006; In preparation, Sun et al. Chapter four; In preparation).

According to grower observations, there could be greater infestation in dryland wheat fields than in those that are irrigated, and irrigation is thought to either cause larval mortality directly, or create favorable environmental conditions for natural enemies to attack the larvae. There are no previous studies on the effects of irrigation on larval survival in wheat fields. In this paper, we report on overall infestation and larval survival

of wheat stem sawflies in dryland and irrigated wheat fields from locations in Montana over three years.

## Materials and Methods

### 2005 Field Experiments

In the summer of 2005, paired, adjacent dryland and irrigated spring wheat fields were selected in Post Experimental Farm at Bozeman, Western Triangle Agricultural Research Center (WTARC) at Conrad, and Southern Agricultural Research Center (SARC) at Huntley, Montana, to compare sawfly infestation and larval survival in field experiments (Figure 6.1 and Table 6.1). The wheat fields chosen for this study were planted with the same variety of spring wheat at each location, and received the same agricultural management, except that about 15 cm of supplemental water were applied to the irrigated fields in July using sprinklers. The spring wheat varieties grown in Bozeman, Conrad, and Huntley were ‘Reeder’, ‘McNeal’, and ‘Scholar’, respectively. Sawfly infestation was achieved using field cages. Three (530  $\mu\text{m}$ ) mesh cages (60  $\times$  20  $\times$  60 cm) were randomly assigned to four rows of wheat plants near the edge of field, after the plants had commenced stem elongation (Zadoks 32-40). 25 female and 10 male adults, reared from sawfly-cut stems in the laboratory, were released into each cage, and held for 7 days to allow mating and oviposition before the cage was removed. The areas that had been infested were flagged along the boundary, and 15 wheat plants were randomly collected in each infested area three times during the wheat growing season. The samples were collected at approximately 20-day intervals at each site. Stems in the

collected wheat samples were dissected with a scalpel in the laboratory. Infestation was recorded as the percentage of stems containing larvae out of the total processed wheat stems in the final sample before harvest. Survival was recorded by counting the number of surviving larvae out of the total larvae found in the processed wheat stems for each sampling event. Parasitoid attack and fungal infection were recorded, because they are the two major biological lethal factors contributing to the larval mortality in the growing crop. Other lethal factors, mainly mechanical damage and desiccation, were also recorded. Colonized larval cadavers with obvious fungal mycelia were individually placed in disposable sterile centrifuge tubes (2ml) for subsequent fungal isolation.

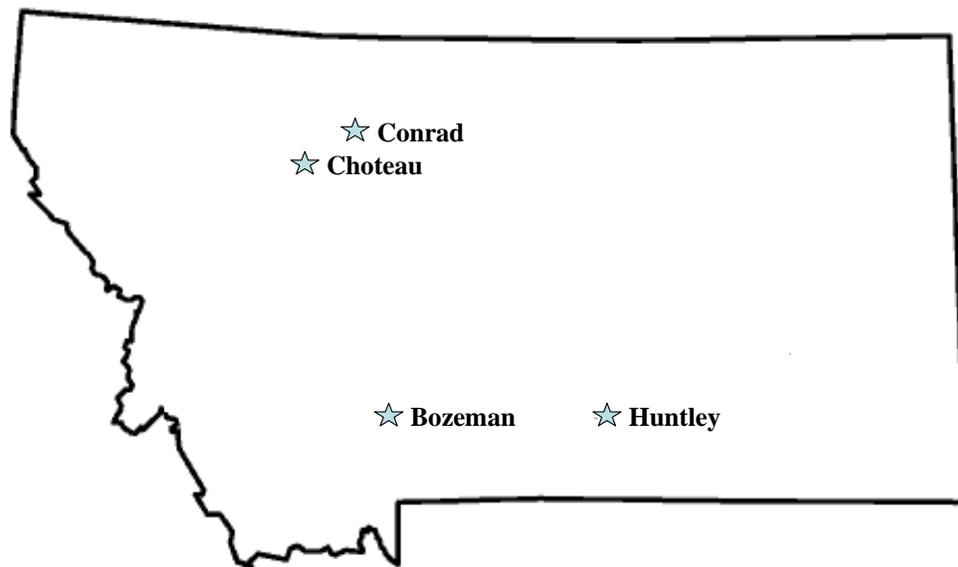


Figure 6.1. Location of field sites used to assess the survival of wheat stem sawfly in Montana.

Table 6.1. Summary of field sites selected for study of wheat stem sawfly survival.

Year	Field sites	Field type	Wheat type	Wheat variety	Irrigation type
2005	Bozeman site	Dryland	Spring wheat	Reeder	---
		Irrigated	Spring wheat	Reeder	Sprinkler
	Conrad site	Dryland	Spring wheat	McNeal	---
		Irrigated	Spring wheat	McNeal	Sprinkler
	Huntley site	Dryland	Spring wheat	Scholar	---
		Irrigated	Spring wheat	Scholar	Sprinkler
2006	Choteau site	Dryland	Winter wheat	Jagelene	---
		Irrigated	Winter wheat	Jagelene	Sprinkler
	Conrad site	Dryland	Winter wheat	NuHorizon	---
		Irrigated	Winter wheat	NuHorizon	Sprinkler
2007	Choteau site A	Dryland	Winter wheat	Jagelene	---
		Irrigated	Winter wheat	Jagelene	Flood
	Choteau site B	Dryland	Winter wheat	Neeley	---
		Irrigated	Winter wheat	Neeley	Flood
	Conrad site A	Dryland	Winter wheat	NuHorizon	---
		Irrigated	Winter wheat	NuHorizon	Sprinkler
	Conrad site B	Dryland	Winter wheat	NuHorizon	---
		Irrigated	Winter wheat	NuHorizon	Sprinkler

### 2006 Field Surveys

In the summer of 2006, paired, adjacent dryland and irrigated winter wheat fields were selected at Choteau and Conrad, Montana, to compare sawfly infestation and larval survival in field surveys (Figure 6.1 and Table 6.1). The wheat varieties were ‘Jagelene’ and ‘NuHorizon’ at Choteau and Conrad, respectively. In July, the irrigated wheat fields received 15 cm of supplemental water by sprinkler delivery at Choteau and Conrad. There were naturally-occurring sawfly populations at both sites. Samples were collected each week after the plants reached stem elongation. There were 8 sampling events in each field during the wheat growing season. For each sampling event, three wheat plants were taken from each of the five randomly selected points among the rows of plants near the edge of the field, and those selected points covered an area more than 300 m long and 15

m wide. Plant density was also assessed by counting the number of stems in a 0.3-meter section of row at each of the 5 randomly selected points. The wheat samples were processed in the laboratory using the same method as for the field experiments in 2005.

Wheat residue containing diapausing larvae in stubs were collected from the wheat fields after harvest, in October (9<sup>th</sup> sampling event) and were also collected in the following April (10<sup>th</sup> sampling event), after overwintering. The stubs were readily identified by the characteristic, precisely cut stems with frass plugs at the apex. For each postharvest sampling event, 20 stubs were collected from each of the 5 randomly selected points among the stubble rows near the edge of the field. The stubs were dissected in the laboratory using a scalpel, and larval survival in the stubs was recorded by counting the number of surviving larvae out of all larvae found in the stubs. Colonized larvae with obvious fungal mycelia were stored separately in disposable sterile centrifuge tubes (2ml) for subsequent fungal isolation.

### 2007 Field Surveys

In the summer of 2007, paired, adjacent dryland and irrigated winter wheat fields were selected at two sites in Choteau and two sites in Conrad, Montana, to compare sawfly infestation and larval survival in field surveys (Figure 6.1 and Table 6.1). The wheat varieties were ‘Jagalene’ and ‘Neeley’ for site A and site B at Choteau, respectively, and the wheat variety was ‘NuHorizon’ for both sites at Conrad. The irrigated fields received 15 cm of supplemental water by flooding at Choteau and by sprinkler delivery at Conrad in July, respectively. There were naturally-occurring sawfly

populations infesting the wheat plants at both sites. The sampling protocol and data collection were identical to the methods for the field surveys in 2006.

#### Fungal Isolation and Identification

Fungal-colonized larval cadavers were individually surface-sterilized by immersion in 80% ethanol for 5 minutes and then dried under a filtered air flow for 20 minutes. The larval cadavers were then plated on water agar supplemented with 50 µg chloramphenicol per ml. After three days incubation at 23 °C, a mycelial tip from each fungal isolate growing out of the larval cadavers was transferred to a potato dextrose agar (PDA) slant for preliminary identification to genus (Barnett and Hunter 1972). Isolates of *Fusarium* were prepared using single conidia techniques on PDA and carnation leaf agar slants (Nelson et al. 1983). The identification of each *Fusarium* species isolated was confirmed by the *Fusarium* Research Center at Pennsylvania State University.

#### Statistical Analysis

Arcsine square-root transformations were performed on all percentage data for stem infestation and larval survival before analysis. For the field experiments in 2005, data for stem infestation and larval survival between dryland and irrigated field at each site were analyzed by analysis of variance (ANOVA) (PROC MIXED, SAS Institute 2001). For the field surveys in 2006 and 2007, a student's t-test was used to make pairwise comparisons in stem infestation, larval survival, and plant density between dryland and irrigated fields at each site (PROC TTEST, SAS Institute 2001).

## Results

### 2005 Field Experiments

In the field experiments during the summer of 2005, stem infestation in dryland and irrigated wheat fields was 20.1% and 24.5% in Bozeman, 26.5% and 34.6% in Conrad, and 43.1% and 45.8% in Huntley, respectively, and there were no significant differences in stem infestation between dryland and irrigated wheat fields (Bozeman -  $F = 0.64$ ,  $df = 1$ ,  $P = 0.507$ ; Conrad -  $F = 4.91$ ,  $df = 1$ ,  $P = 0.151$ ; Huntley -  $F = 17.31$ ,  $df = 1$ ,  $P = 0.053$ ) (Figure 6.2). Larval survival was nearly 100% in growing plants in both dryland and irrigated fields at the three sites for the 1<sup>st</sup> sampling event (Figure 6.3, Figure 6.4, and Figure 6.5). For the 2<sup>nd</sup> sampling event, larval survival in the dryland and irrigated wheat fields was 98.1% and 100.0% at Bozeman, 38.2% and 42.0% at Conrad, and 100.0% and 90.4% at Huntley, respectively. There was a significant difference in overall larval survival between dryland and irrigated wheat fields at Huntley ( $F = 31.13$ ,  $df = 1$ ,  $P = 0.031$ ), but not at Bozeman and Conrad (Bozeman -  $F = 1.00$ ,  $df = 1$ ,  $P = 0.422$ ; Conrad -  $F = 0.21$ ,  $df = 1$ ,  $P = 0.695$ ). A large percentage of the larvae were parasitized in the dryland (61.8%) and irrigated (53.6%) wheat fields at Conrad, but no significant difference was evident ( $F = 0.78$ ,  $df = 1$ ,  $P = 0.469$ ) (Figure 6.4). For the 3<sup>rd</sup> sampling event, larval survival in the dryland and irrigated wheat fields was 74.1% and 92.1% at Bozeman, 13.4% and 14.7% at Conrad, and 94.0% and 64.2% at Huntley, respectively. A significant difference was evident between dryland and irrigated fields at Huntley ( $F = 56.76$ ,  $df = 1$ ,  $P = 0.017$ ), but not at Bozeman and Conrad (Bozeman -  $F =$

3.17,  $df = 1$ ,  $P = 0.217$ ; Conrad -  $F = 0.00$ ,  $df = 1$ ,  $P = 0.963$ ). The percentage of parasitized larvae in the irrigated field (31.5%) was significantly greater than in the dryland field (0%) at Huntley, which contributed to the significant difference in overall larval survival ( $F = 312.44$ ,  $df = 1$ ,  $P = 0.003$ ) (Figure 6.5). The percentage of fungal-colonized larvae in the dryland and irrigated fields at Huntley was 5.2% and 1.9%, respectively, and the difference was significant ( $F = 33.25$ ,  $df = 1$ ,  $P = 0.028$ ).

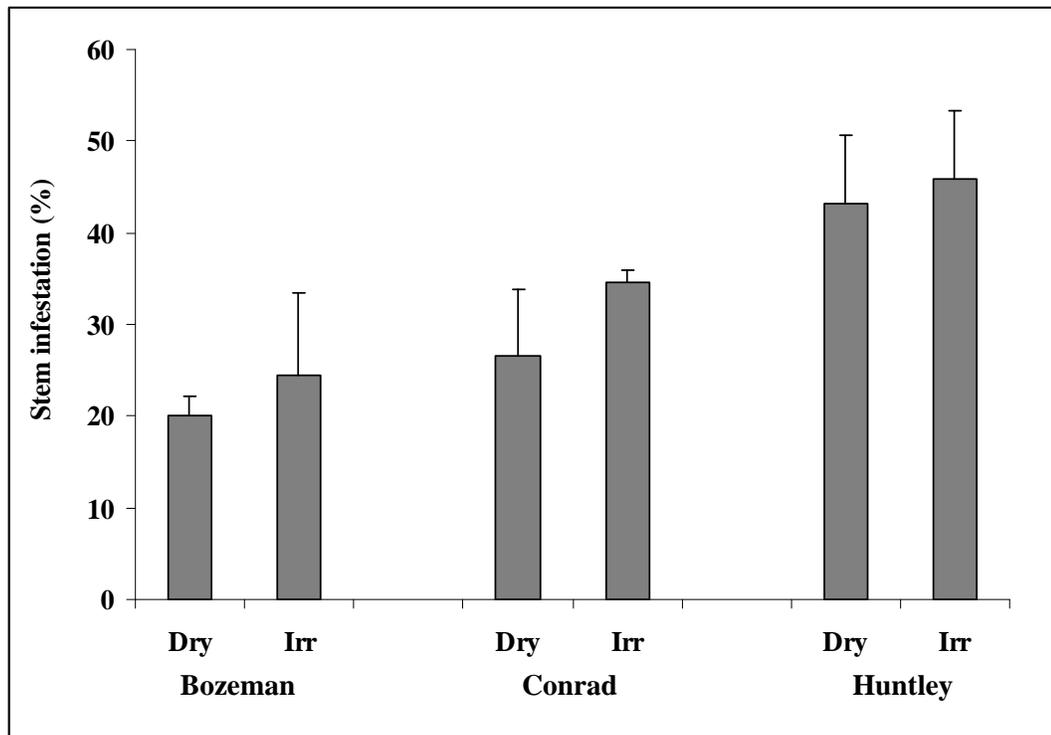


Figure 6.2. The percentage of stems infested by wheat stem sawflies in both dryland and irrigated wheat fields in samples collected near Bozeman, Conrad, and Huntley, MT, in 2005; Dry - dryland, Irr - irrigated.

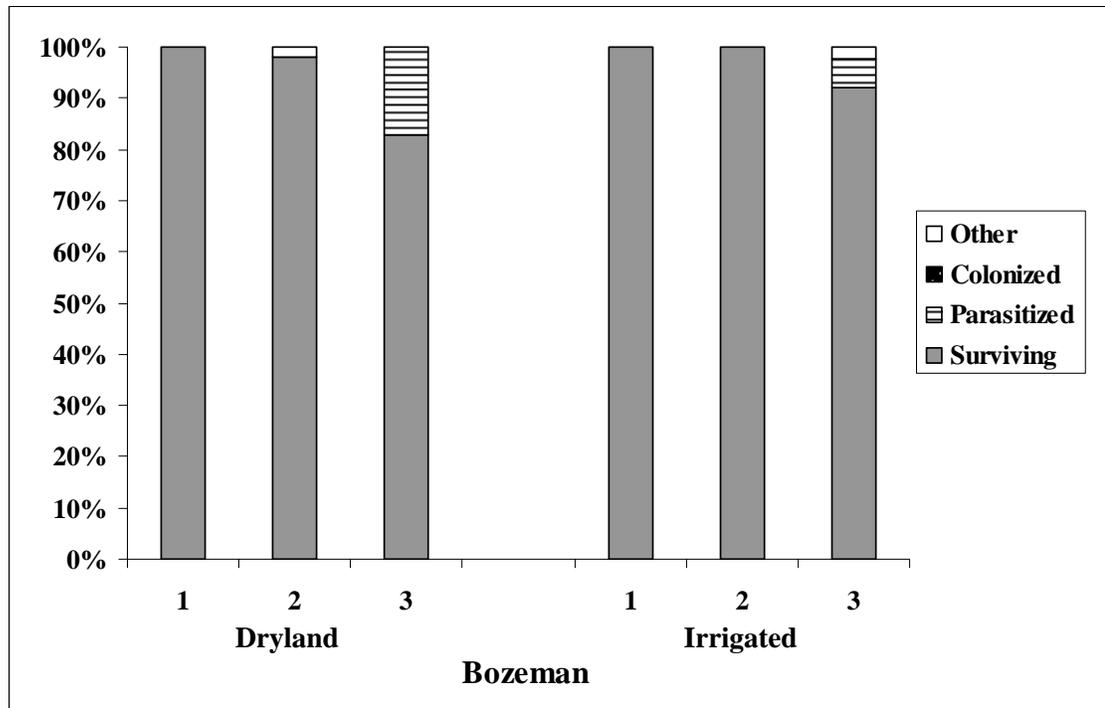


Figure 6.3. The percentage of surviving, parasitized, and fungal-colonized larvae, or larvae killed by other factors in dryland and irrigated wheat fields at Bozeman in 2005. Along the x-axis 1, 2, and 3 are the sampling events; these occurred on Julian date 193, 215, and 237 respectively. The samples consisted of green wheat plants containing developing sawfly larvae.

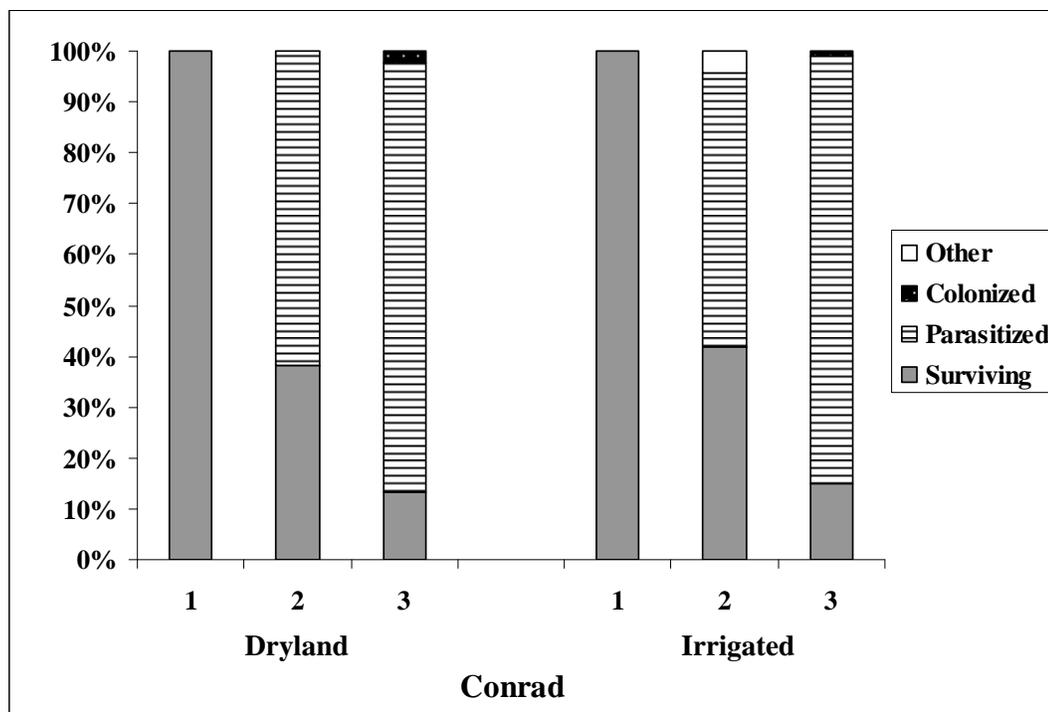


Figure 6.4. The percentage of surviving, parasitized, and fungal-colonized larvae, or larvae killed by other factors in dryland and irrigated wheat fields at Conrad in 2005. Along the x-axis 1, 2, and 3 are the sampling events; these occurred on Julian date 188, 209, and 230 respectively. The samples consisted of green wheat plants containing developing sawfly larvae.

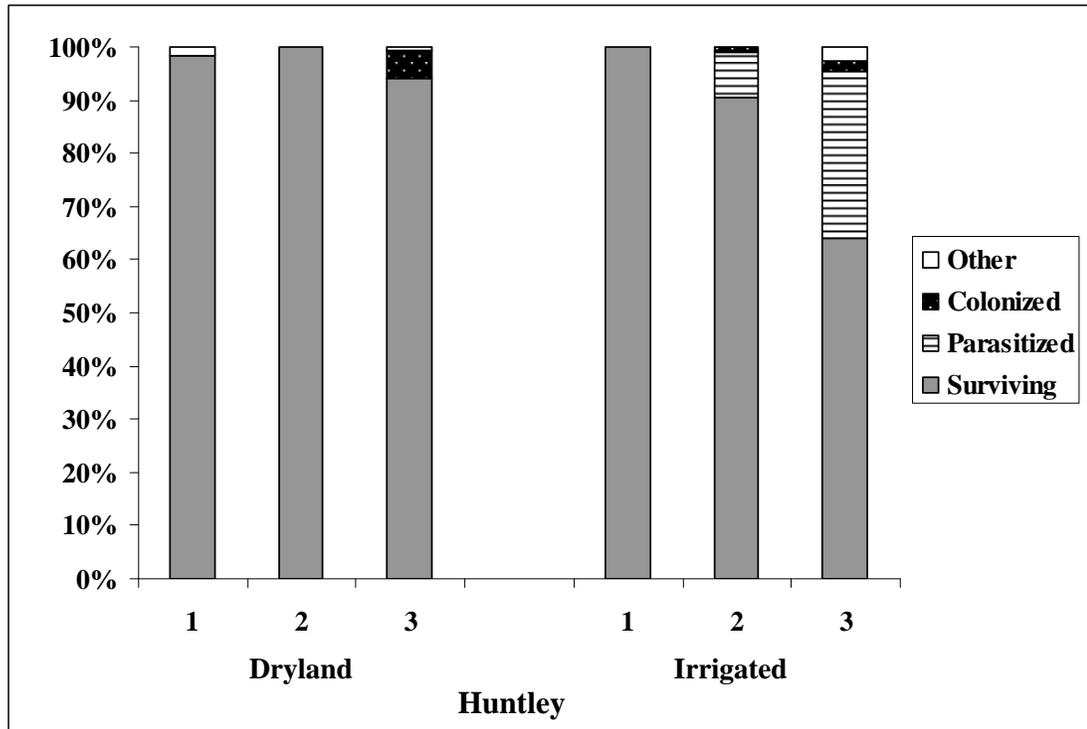


Figure 6.5. The percentage of surviving, parasitized, and fungal-colonized larvae, or larvae killed by other factors in dryland and irrigated wheat fields at Huntley in 2005. Along the x-axis 1, 2, and 3 are the sampling events; these occurred on Julian date 182, 203, and 222 respectively. The samples consisted of green wheat plants containing developing sawfly larvae.

### 2006 Field Surveys

In the summer of 2006, stem infestation in dryland and irrigated fields was 74.9% and 43.9% at Choteau, and 57.2% and 53.4% at Conrad, respectively, and there was a significant difference in stem infestation between dryland and irrigated wheat fields at Choteau ( $t = 4.15$ ,  $df = 8$   $P = 0.003$ ), but not at Conrad ( $t = 0.67$ ,  $df = 8$   $P = 0.523$ ) (Figure 6.6). There was no evident mortality in samples containing developing larvae in either the dryland or the irrigated fields at Choteau and Conrad for the first 4 sampling events (Figure 6.7 and Figure 6.8). For the 5<sup>th</sup> through the 8<sup>th</sup> sampling events, there were

no significant differences in overall larval survival between dryland and irrigated fields at Choteau ( $t = -2.20, 0.32, -0.87, \text{ and } -0.14$  for the 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup>, and 8<sup>th</sup> sampling events, respectively,  $df = 8, P > 0.05$ ) or at Conrad ( $t = -2.14, 0.32, 0.16, \text{ and } -0.55$  for the 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup>, and 8<sup>th</sup> sampling event, respectively,  $df = 8, P > 0.05$ ). The percentage of parasitized larvae at the dryland and irrigated fields at Choteau was 13.6% and 13.0%, 5.6% and 11.5%, 14.0% and 14.3%, 19.6% and 13.0%, respectively, for the 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup>, and 8<sup>th</sup> sampling event, but no significant difference in parasitism was evident for any sampling events ( $t = 2.14, 0.01, 0.56, \text{ and } 1.43$  for the 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup>, and 8<sup>th</sup> sampling event, respectively,  $df = 8, P > 0.05$ ) (Figure 6.7). The percentage of parasitized larvae at Conrad was 18.5%, 5.6%, 8.8%, and 18.8% in the dryland field, and 0%, 7.9%, 11.1%, and 14.8% in the irrigated field for the 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup>, and 8<sup>th</sup> sampling event, respectively, but a significant difference was evident only for the 5<sup>th</sup> sampling event ( $t = 3.50$  for the 5<sup>th</sup> sampling event,  $df = 8, P = 0.008$ ;  $t = -0.32, -0.16, \text{ and } 0.32$  for the 6<sup>th</sup>, 7<sup>th</sup>, and 8<sup>th</sup> sampling event, respectively,  $df = 8, P > 0.05$ ) (Figure 6.8). The overall mean density of wheat plants in the dryland and irrigated wheat fields was 169.3 and 213.9 stems  $m^{-1}$  at Choteau, and 133.2 and 199.5 stems  $m^{-1}$  at Conrad, respectively. The plant density in the irrigated field was significantly greater than in the dryland field at Choteau ( $t = -4.44, df = 8, P = 0.002$ ) and at Conrad ( $t = -8.54, df = 8, P < 0.001$ ).

For the 9<sup>th</sup> and 10<sup>th</sup> sampling events after harvest, a significant difference was evident for survival of diapausing larvae in dryland (83.8%) and irrigated (71.4%) fields at Choteau for the 10<sup>th</sup> sampling event ( $t = 3.34, df = 6, P = 0.014$ ), but not for the 9<sup>th</sup> sampling event ( $t = 0.50, df = 6, P = 0.627$ ). There was a significantly greater percentage

of larvae killed by other factors in the irrigated field (11.1%) than in the dryland field (0.6%) at Choteau for the 10<sup>th</sup> sampling event ( $t = -5.66$ ,  $df = 6$ ,  $P = 0.001$ ). This contributes to the greater overall mortality of diapausing larvae in the irrigated field. The percentage of fungal-colonized larvae in the dryland and irrigated fields at Choteau was 5.1% and 7.1% in the 9<sup>th</sup> sampling event, and 14.4% and 16.7% in the 10<sup>th</sup> sampling event, respectively, and no significant difference was evident at either sampling event (9<sup>th</sup> sampling event -  $t = -0.11$ ,  $df = 6$ ,  $P = 0.912$ ; 10<sup>th</sup> sampling event -  $t = -1.47$ ,  $df = 6$ ,  $P = 0.192$ ). The percentage of fungal-colonized larvae at Conrad increased from 4.2% to 11.9% in the dryland field, and from 5.3% to 13.4% in the irrigated field between the 9<sup>th</sup> and 10<sup>th</sup> sampling events, but no significant difference between dryland and irrigated fields was evident for either sampling event (9<sup>th</sup> sampling event -  $t = 0.77$ ,  $df = 6$ ,  $P = 0.495$ ; 10<sup>th</sup> sampling event -  $t = -1.08$ ,  $df = 6$ ,  $P = 0.320$ ). There was no significant difference in overall larval survival between the dryland and irrigated fields at Conrad for the 9<sup>th</sup> and 10<sup>th</sup> sampling events (9<sup>th</sup> sampling event -  $t = -0.70$ ,  $df = 6$ ,  $P = 0.535$ ; 10<sup>th</sup> sampling event -  $t = 0.74$ ,  $df = 6$ ,  $P = 0.485$ ) (Figure 6.8).

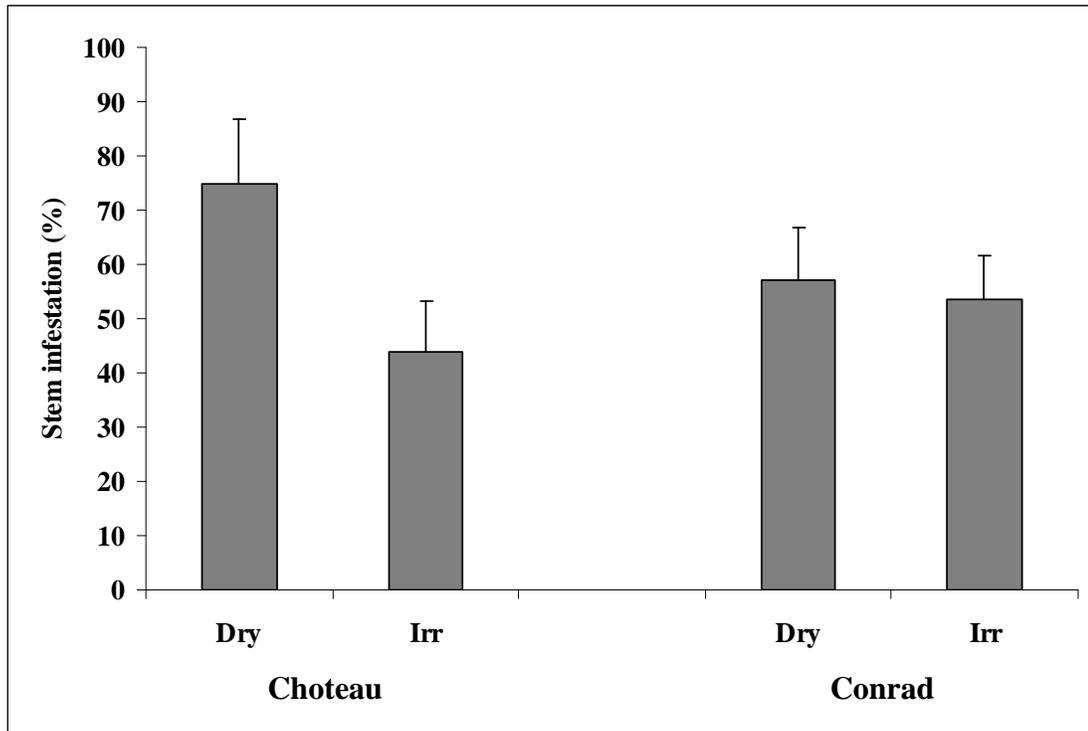


Figure 6.6. The percentage of stems infested by wheat stem sawflies in both dryland and irrigated wheat fields at Choteau and Conrad in 2006; Dry - dryland, Irr - irrigated.

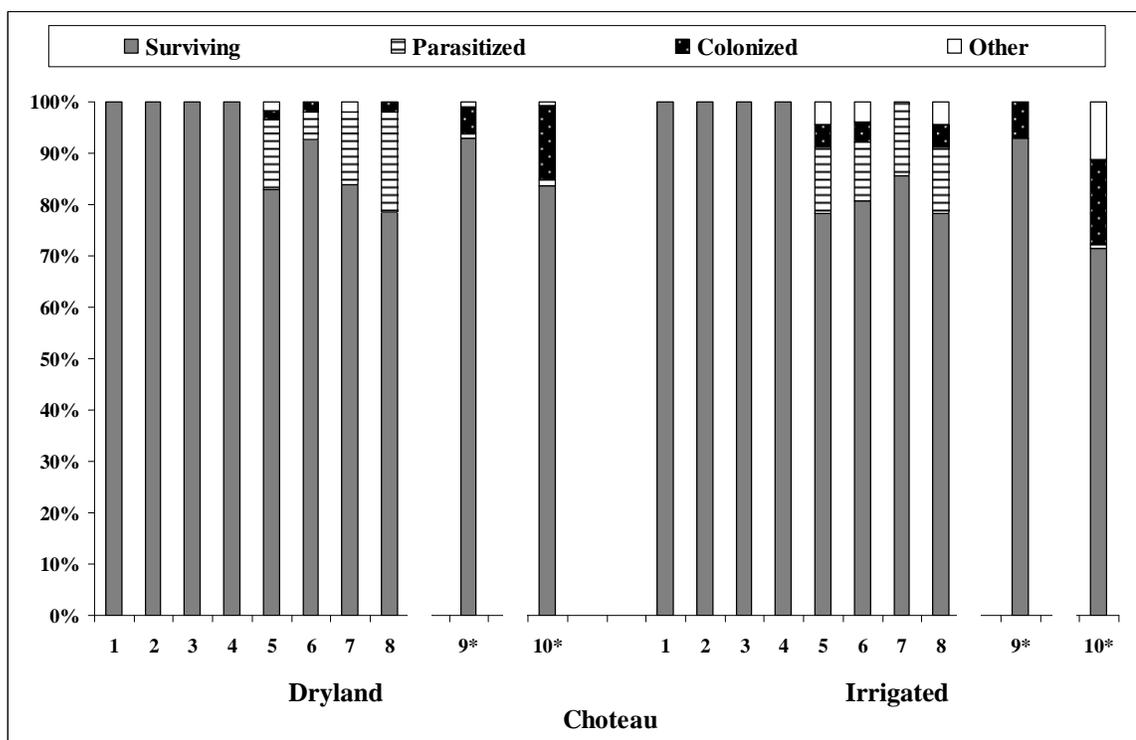


Figure 6.7. The percentage of surviving, parasitized, and fungal-colonized larvae, or larvae killed by other factors in dryland and irrigated wheat fields at Choteau in 2006. Along the x-axis, the number of 1-10 indicates the sampling event; sampling events 1-8 occurred between Julian day 166 and 215 at one week intervals in 2006 and the samples consisted of green wheat plants containing developing sawfly larvae; sampling events 9\* and 10\* occurred on Julian day 296 in 2006 and Julian day 108 in 2007 respectively, the samples consisted of sawfly-cut stems containing diapausing larvae after harvest and after overwintering, respectively.

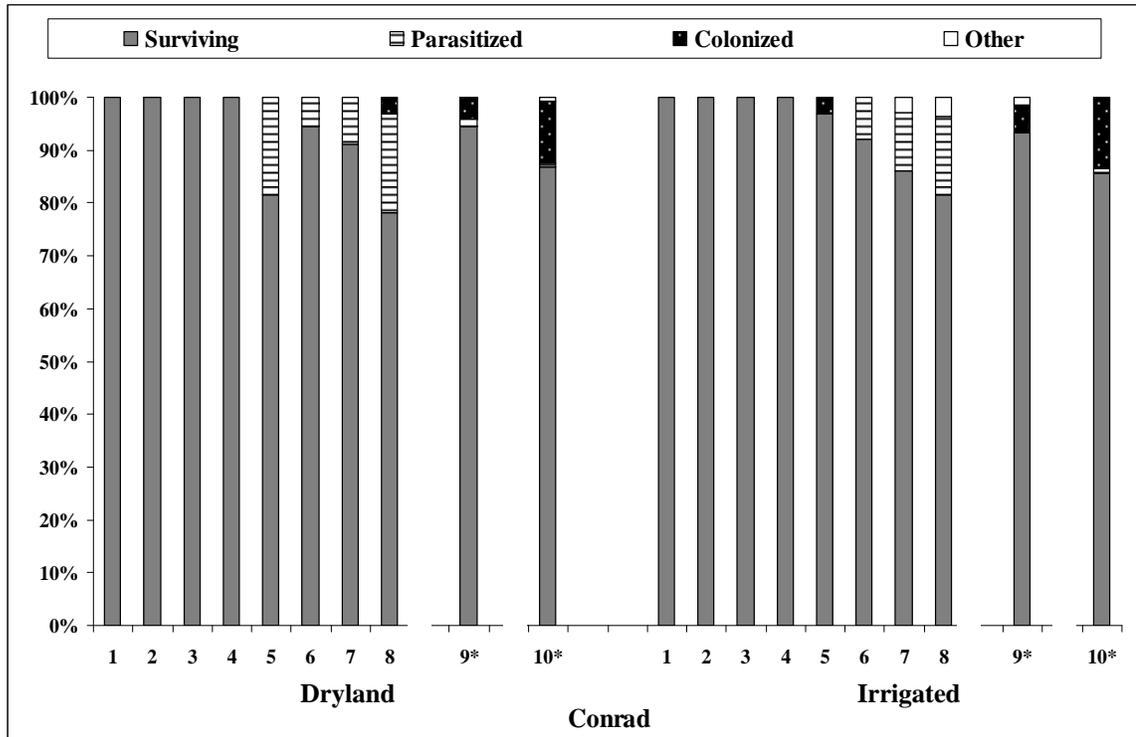


Figure 6.8. The percentage of surviving, parasitized, and fungal-colonized larvae, or larvae killed by other factors in dryland and irrigated wheat fields at Conrad in 2006. Along the x-axis, the number of 1-10 indicates the sampling event; sampling events 1-8 occurred between Julian day 166 and 215 at one week intervals in 2006 and the samples consisted of green wheat plants containing developing sawfly larvae; sampling events 9\* and 10\* occurred on Julian day 296 in 2006 and Julian day 108 in 2007 respectively, the samples consisted of sawfly-cut stems containing diapausing larvae after harvest and after overwintering, respectively.

### 2007 Field Surveys

In the summer of 2007, stem infestation in dryland and irrigated fields was 98.6% and 61.9% for site A, and 39.6% and 21.7% for site B, respectively, at Choteau. A significant difference in stem infestation was evident for both site A and site B (site A -  $t = 9.44$ ,  $df = 8$ ,  $P < 0.001$ ; site B -  $t = 2.42$ ,  $df = 8$ ,  $P = 0.042$ ) (Figure 6.9). Stem infestation in dryland and irrigated fields was 65.8% and 60.8% for site A, and 83.3% and 71.2% for site B, respectively, at Conrad, but no significant difference in stem

infestation was evident at either site (site A -  $t = 0.66$ ,  $df = 8$ ,  $P = 0.530$ ; site B -  $t = 1.56$ ,  $df = 8$ ,  $P = 0.157$ ) (Figure 6.9). Similar patterns in larval mortality were observed for the first 4 sampling events across the 4 sites at Choteau and Conrad, with almost 100% survival of developing larvae in both dryland and irrigated wheat fields (Figure 6.10, Figure 6.11, Figure 6.12, and Figure 6.13).

For the interval spanning the 5<sup>th</sup> through 8<sup>th</sup> sampling events, there were no significant differences in larval survival between the dryland and irrigated fields at either of the selected sites at Choteau and at Conrad, except for site A at Choteau for the 7<sup>th</sup> and 8<sup>th</sup> sampling events ( $t = 5.73$  and  $3.45$  for the 7<sup>th</sup> and 8<sup>th</sup> sampling event, respectively,  $df = 8$ ,  $P < 0.05$ ). Parasitism was greater in irrigated fields than in dryland fields for both sites at Choteau, and significant difference was evident for each sampling event at site A ( $t = -7.38$ ,  $-6.17$ ,  $-5.62$ , and  $-5.07$  for the 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup>, and 8<sup>th</sup> sampling events, respectively,  $df = 8$ ,  $P < 0.05$ ), but not at site B ( $t = -0.10$ ,  $-0.01$ ,  $-0.96$ , and  $-0.87$  for the 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup>, and 8<sup>th</sup> sampling event, respectively,  $df = 8$ ,  $P > 0.05$ ) (Figure 6.10 and Figure 6.11). Opposite trends were apparent for the percentage of fungal-colonized developing larvae in the dryland and irrigated fields at Choteau, these were 0% and 4.8% respectively at site A for the 7<sup>th</sup> sampling event, and 4.2% and 0% respectively at site B for the 8<sup>th</sup> sampling event ( $t = -3.97$  for the 7<sup>th</sup> sampling event at site A,  $df = 8$ ,  $P = 0.004$ ;  $t = 1.00$  for the 8<sup>th</sup> sampling event at site B,  $df = 8$ ,  $P = 0.346$ ). The percentage of parasitized developing larvae in the dryland field (17.5%) was significantly higher than in the irrigated field (0%) for site B at Conrad for the 8<sup>th</sup> sampling event ( $t = 42.19$ ,  $df = 8$ ,  $P < 0.001$ ) (Figure 6.13). There was a significant difference in the percentage of fungal-

colonized larvae between the dryland field (12.5%) and the irrigated field (2.0%) for site A at Conrad for the 8<sup>th</sup> sampling event ( $t = -6.37$ ,  $df = 8$ ,  $P < 0.001$ ). The overall mean density of wheat plants in the dryland and irrigated wheat fields was 142.0 and 164.2 stems  $m^{-1}$  for site A at Choteau, and 113.5 and 154.2 stems  $m^{-1}$  for site B at Choteau, and 140.4 and 182.8 stems  $m^{-1}$  for site A at Conrad, and 145.1 and 176.8 stems  $m^{-1}$  for site B at Conrad, respectively. The plant density in irrigated field was significantly higher than in dryland field at all the sampled field sites ( $t = -2.80$ ,  $-3.29$ ,  $-4.78$ , and  $-3.85$  for Choteau site A, Choteau site B, Conrad site A, and Conrad site B, respectively,  $df = 8$ ,  $P < 0.05$ ).

For the 9<sup>th</sup> sampling event, the percentage of fungal-colonized larvae in diapause for the dryland and irrigated fields was 9.0% and 10.0% for site A, and 7.0% and 4.7% for site B at Choteau, respectively, but no significant difference was evident at either site (site A -  $t = -0.45$ ,  $df = 8$ ,  $P = 0.666$ ; site B -  $t = 1.20$ ,  $df = 8$ ,  $P = 0.264$ ) (Figure 6.10 and Figure 6.11). The overall survival of diapausing larvae in dryland and irrigated wheat fields was not significantly different at Choteau for either site A or site B (site A -  $t = 2.37$ ,  $df = 8$ ,  $P = 0.052$ ; site B -  $t = -1.20$ ,  $df = 8$ ,  $P = 0.265$ ). The percentage of surviving larvae in diapause in the dryland and irrigated fields was 92.0% and 82.0%, and 93.0 and 81.0%, for site A and site B, respectively, at Conrad for the 9<sup>th</sup> sampling event, and a significant difference in larval survival was evident for site B ( $t = 2.74$ ,  $df = 8$ ,  $P = 0.025$ ), but not for site A ( $t = 2.14$ ,  $df = 1$ ,  $P = 0.065$ ). The percentage of fungal-colonized larvae was greater for both sites at Conrad for the 9<sup>th</sup> sampling event, but no significant difference was detected for either site (site A -  $t = -1.03$ ,  $df = 8$ ,  $P = 0.332$ ; site B -  $t =$

-2.19,  $df = 8$ ,  $P = 0.060$ ). A greater percentage of larvae killed by other factors were found in the irrigated fields than in the dryland fields for both sites at Conrad for the 9<sup>th</sup> sampling event, and a significant difference was evident for site B ( $t = -2.38$ ,  $df = 8$ ,  $P = 0.045$ ), but not for site A ( $t = -1.59$ ,  $df = 8$ ,  $P = 0.150$ ).

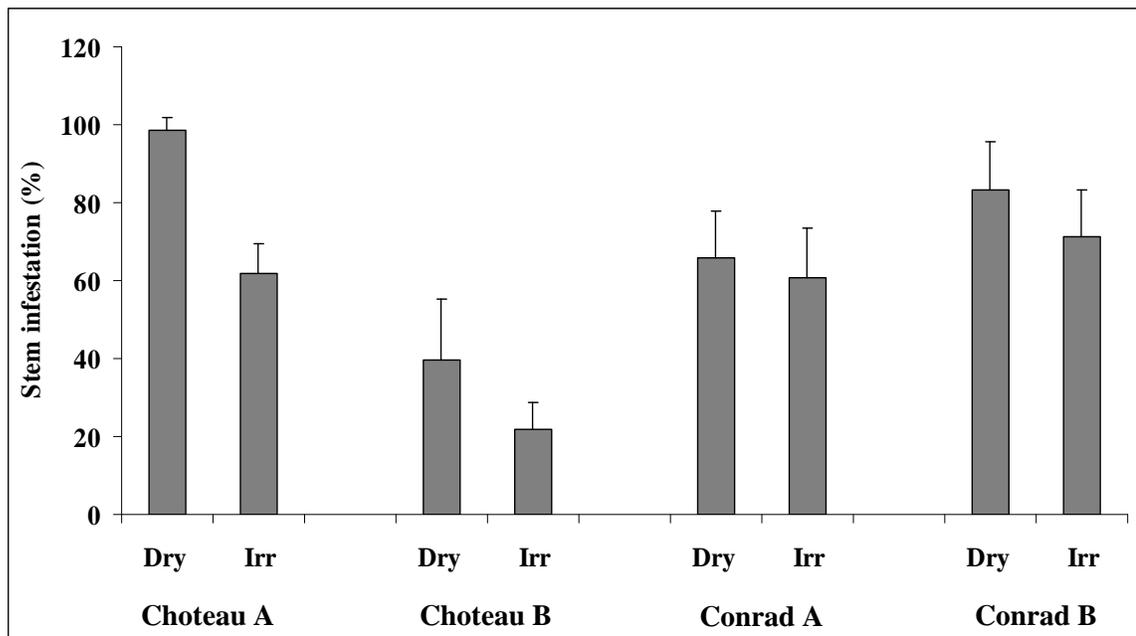


Figure 6.9. The percentage of stems infested by wheat stem sawflies in both dryland and irrigated wheat fields at Choteau and Conrad in 2007; A and B indicate site A and site B respectively; Dry - dryland, Irr - irrigated.

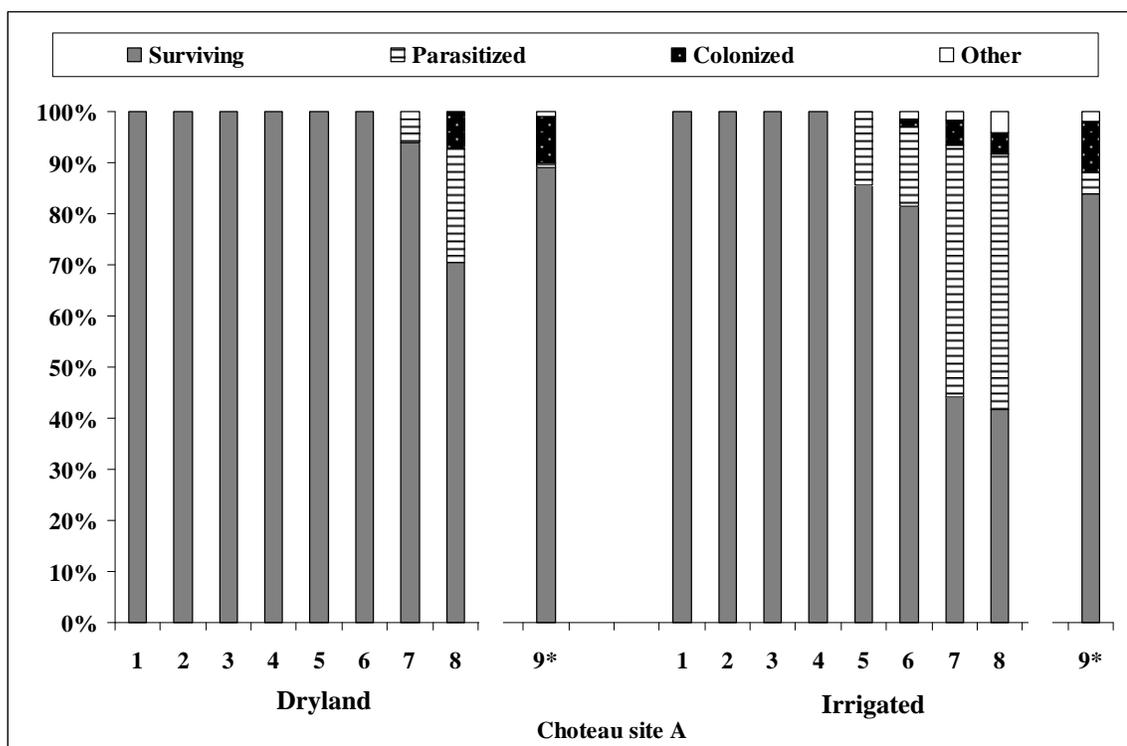


Figure 6.10. The percentage of surviving, parasitized, and fungal-colonized larvae, or larvae killed by other factors in dryland and irrigated wheat fields at Choteau site A in 2007.

Along the x-axis, the number of 1-9 indicates the sampling event; sampling events 1-8 occurred between Julian day 165 and 214 at one week interval in 2007 and the samples consisted of green wheat plants containing developing sawfly larvae; sampling event 9\* occurred on Julian day 296 in 2007, the samples consisted of sawfly-cut stems containing diapausing larvae after harvest.

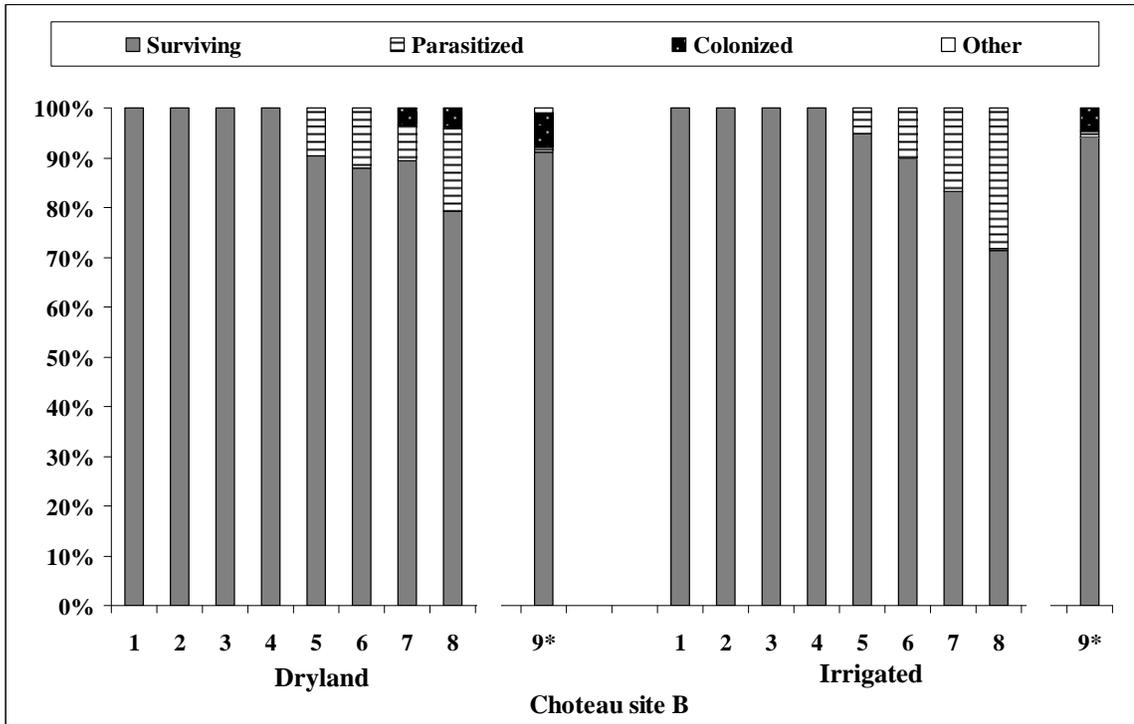


Figure 6.11. The percentage of surviving, parasitized, and fungal-colonized larvae, or larvae killed by other factors in both dryland and irrigated wheat fields at Choteau site B in 2007.

Along the x-axis, the number of 1-9 indicates the sampling event; sampling events 1-8 occurred between Julian day 165 and 214 at one week interval in 2007 and the samples consisted of green wheat plants containing developing sawfly larvae; sampling event 9\* occurred on Julian day 296 in 2007, the samples consisted of sawfly-cut stems containing diapausing larvae after harvest.

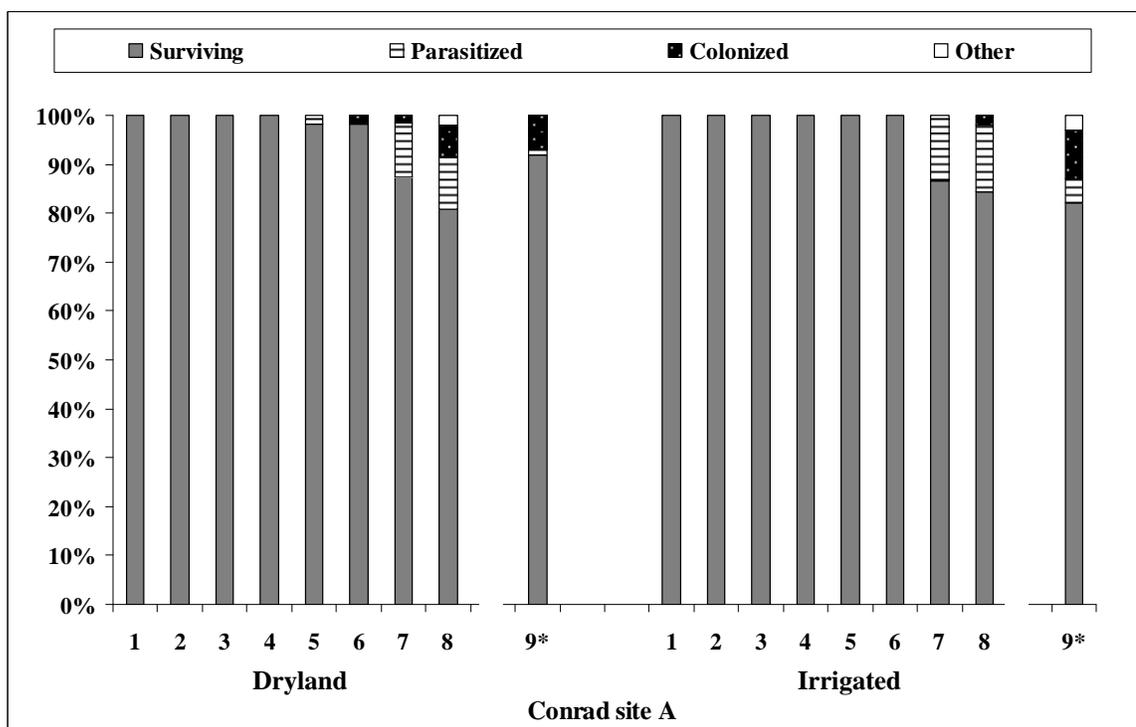


Figure 6.12. The percentage of surviving, parasitized, and fungal-colonized larvae, or larvae killed by other factors in dryland and irrigated wheat at Conrad site A in 2007. Along the x-axis, the number of 1-9 indicates the sampling event; sampling events 1-8 occurred between Julian day 165 and 214 at one week interval in 2007 and the samples consisted of green wheat plants containing developing sawfly larvae; sampling event 9\* occurred on Julian day 296 in 2007, the samples consisted of sawfly-cut stems containing diapausing larvae after harvest.

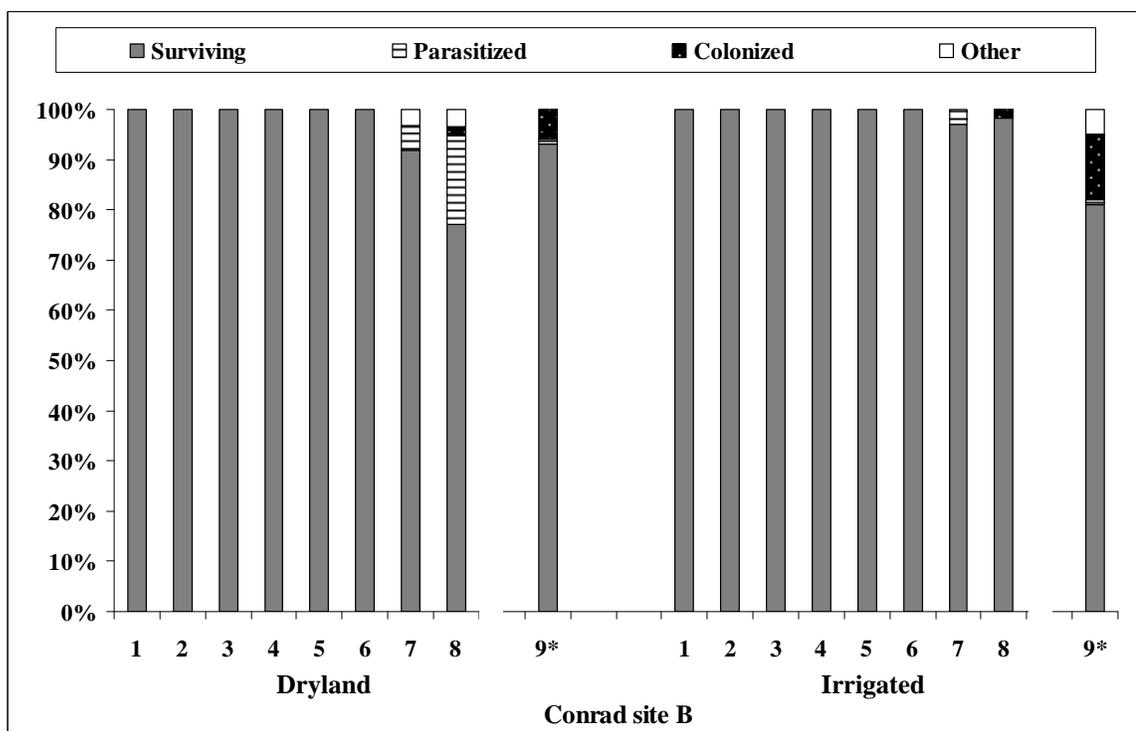


Figure 6.13. The percentage of surviving, parasitized, and fungal-colonized larvae, or larvae killed by other factors in dryland and irrigated wheat fields at Conrad site B in 2007.

Along the x-axis, the number of 1-9 indicates the sampling event; sampling events 1-8 occurred between Julian day 165 and 214 at one week interval in 2007 and the samples consisted of green wheat plants containing developing sawfly larvae; sampling event 9\* occurred on Julian day 296 in 2007, the samples consisted of sawfly-cut stems containing diapausing larvae after harvest.

### Fungal Isolation and Identification

The frequency of occurrence for the fungal species isolated from colonized larval cadavers in the field in 2005, 2006, and 2007, is shown in Figure 6.14. A group of *Fusarium* spp. were the dominant fungi isolated across all sites, and the species included *F. culmorum*, *F. pseudograminearum*, *F. acuminatum*, *F. avenaceum*, plus some unidentified *Fusarium* species. Isolates of *Paecilomyces* spp., *Cunninghamella* spp., *Monilia* spp., and *Penicillium* spp., were also obtained from larval cadavers.

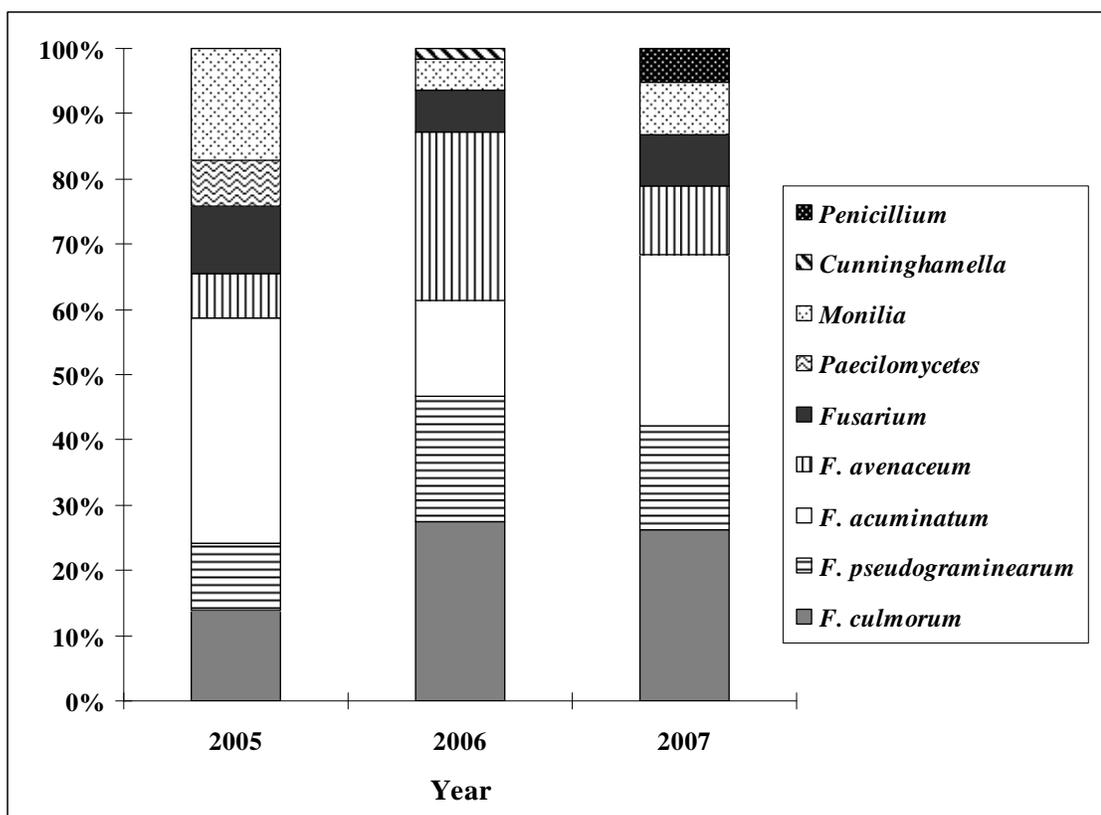


Figure 6.14. Relative occurrence of fungal species isolated from colonized larval cadavers of wheat stem sawfly in the field experiments and field surveys during 2005, 2006, and 2007.

The number of fungal isolates obtained from colonized larvae was 29, 62, and 38 in 2005, 2006, and 2007, respectively.

## Discussion

The field data from experiments or surveys for three years showed that there were no obvious differences for stem infestation and larval survival between dryland and irrigated wheat fields. Infestation was numerically higher in the dryland fields than in the corresponding irrigated fields across all the sites in the field surveys for both 2006 and 2007, which seemed consistent with grower observations (Figure 6.6 and Figure 6.9). However, greater stem infestation was recorded in the irrigated fields than in corresponding dryland fields across all sites in the field experiment of 2005, which indicates that larval sawflies actually survived better in the irrigated fields than dryland fields once they gained access to the wheat plants (Figure 6.9). Overall, there were some fluctuations in larval mortality caused by parasitoids, fungal attack, and other factors in both dryland and irrigated fields, but no conclusive trend in larval survival could be attributed to these, and all the variability in lethal factors can be attributed to specific, localized issues. For example, the parasitism percentage was greater in irrigated fields than in dryland fields during the summer for both sites at Choteau in 2007, while the parasitism percentage showed the opposite trends for both sites at Conrad, with the dryland fields having a greater percentage of parasitized larvae than the corresponding irrigated fields (Figure 6.10, Figure 6.11, Figure 6.12, and Figure 6.13). In the 9<sup>th</sup> sampling event, the percentage of fungal-colonized larvae in diapause was greater for the dryland field (7.0%) than for the irrigated field (4.7%) for site B at Choteau, but it was

higher for the irrigated field (10.0%) than for the dryland field (7.0%) for site A at Conrad (Figure 6.11 and Figure 6.12).

Parasitoid attack and fungal infection, primarily by *Fusarium* spp., were found to be the major lethal factors for developing larvae during the summer, and diapausing larvae during overwintering stage, respectively. The increased pressure exerted by parasitoids as the season progressed can be attributed to the second generation of parasitoids appearing in mid-August. However, additional mortality of diapausing larvae due to parasitoids is quite limited because they are relatively unable to access the sawfly larvae contained within stubs for the brief postharvest interval before temperatures decrease to limit insect activity. This explains the low percentage of parasitism of diapausing larvae inside stubs collected after harvest. *Fusarium* spp. had an important role in overwintering larval mortality, and this scenario was evident in both dryland and irrigated fields. Interestingly, we also conducted a postharvest field survey of mortality in overwintering larvae by collecting stubs from three other locations, including Amsterdam, Havre, and Loring, Montana, in March of 2007. All surveyed locations had greater than 10% mortality in diapausing larvae, with more than 90% caused by *Fusarium* spp., including *F. culmorum*, *F. pseudograminearum*, *F. acuminatum*, *F. avenaceum*, and *F. equiseti* (Sun et al. Chapter one, In preparation). As for other lethal factors, mechanical damage was more prevalent after harvest in irrigated fields than in dryland fields. For example, 5% larval mortality was recorded in the irrigated field at Conrad at site B for the 9<sup>th</sup> sampling event in 2007, while there was almost no larval

mortality caused by mechanical damage at this time in the corresponding dryland field at this site (Figure 6.13).

The grower observations of greater sawfly infestation in dryland fields can be a result of the differences in agricultural practices between dryland and irrigated wheat fields. In the dryland farming areas, alternate-year summer fallow, with minimal or no tillage, was adopted as a method for conserving soil moisture and nitrogen for subsequent crop growth (Blevins and Frye 1993). This crop-fallow system consists of the current growing crop immediately adjacent to idle fields in which the crop of the previous year was located. However, the fallow fields serve as temporary spatial reservoirs for the overwintering sawfly larvae in the wheat residue. This dryland farming system provides near optimal refuge for developing sawfly populations. A 10% annual increase in sawfly infestation was observed in some dryland wheat fields, and near 100% sawfly infestation was recorded in the dryland wheat fields at Choteau in 2007, which probably developed from the annually accumulating sawfly populations in this dryland cropping system. The seeding rate and plant density in the irrigated fields tend to be greater than for dryland fields, which could result in a lower percentage of stem infestation when plants in dryland and irrigated fields are facing similar sawfly pressure. However, overall sawfly infestation should be similar in dryland and irrigated fields because there is no difference in sawfly survival. In addition, the greater density of wheat plants in irrigated fields can also create a dilution of the apparency of sawfly damage when compared to dryland fields before harvest, because there are more available standing stems to prevent the sawfly-cut stems from lodging. In the irrigated wheat fields, the extra water supply enables the

growers to cultivate wheat or other crops on a yearly basis, and a wheat-barley rotation is the most popular agricultural practice in irrigated fields in Montana. Barley is not as suitable as wheat as a host for wheat stem sawfly, and this also could reduce sawfly populations. Other rotations, like wheat-canola, wheat-alfalfa, and wheat-bean, could locally eliminate sawfly populations because these broadleaf crops are not hosts. In addition, using a harrow to fragment infested stubble and subsequently recropping in irrigated wheat fields can significantly reduce the sawfly populations when compared to leaving the wheat residue in the field intact and managing weeds with glyphosate herbicide (Beres and Cárcamo 2006). About 11% larval mortality was observed in mechanically damaged stubble exposed on the soil surface by harrowing and subsequently recropping in the irrigated fields at Choteau in 2006 (Figure 6.7). So, the differences in agricultural practices used for dryland and irrigated wheat fields can explain why greater sawfly infestation could occur in the dryland than in the irrigated wheat fields because the former appears to support increasing sawfly populations yearly, while the latter may suppress sawfly populations each year. However, severe sawfly infestations can also occur in irrigated wheat fields, especially if they are near infested dryland fields, because sawfly adults can migrate from the fallow fields for more than 1 km, and more than 60% stem infestation was also observed in the irrigated fields for both site A and site B at Conrad in 2007 (Figure 6.9)

In summary, there were no obvious differences in sawfly infestation and larval survival for dryland and irrigated wheat fields in the field experiments or field surveys over the three years that these were studied. Parasitoid attack, fungal infection, and

mechanical damage were the major lethal factors to larval wheat stem sawflies. Grower observations of greater sawfly infestation in dryland fields can be due to the differences in annual cropping and other agricultural practices between dryland and irrigated wheat fields. Agricultural practices in the irrigated fields, including rotations and harrowing followed by recropping, may suppress sawfly populations yearly, while alternate-year summer fallow and conservation tillage in the dryland fields may allow increase of sawfly populations on a yearly basis. Other agricultural strategies, such as optimizing spatial arrangements of dryland and irrigated fields over large and contiguous areas, should be evaluated for potential to further suppress populations of the wheat stem sawfly.

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## CHAPTER SEVEN

## CONCLUSIONS AND FUTURE DIRECTIONS

This dissertation has elucidated that, *Fusarium* spp., including *F. culmorum*, *F. pseudograminearum*, *F. acuminatum*, *F. avenaceum*, and *F. equiseti*, were the major factors causing overwintering larval mortality of wheat stem sawfly, and could account for reduction of ten to twenty percent of the overwintering population. These *Fusarium* spp. caused lethal effects in both diapausing larvae in wheat stubble and in actively-feeding larvae in growing winter wheat plants. They also caused decomposed sawfly-cut stems and induced disease symptoms in wheat plants. Deoxynivalenol, produced by *Fusarium* spp. colonizing wheat stems, was toxic to developing larvae, and potential impact on sawfly populations may be underestimated. There were no obvious differences between dryland and irrigated wheat fields for stem infestation or for sawfly mortality caused by *Fusarium* infection. Wheat grower observations of greater sawfly infestation in dryland fields may be due to differences in annual cropping and other agricultural parameters in dryland and irrigated wheat fields.

There are some future projects that could be undertaken to continue to develop these researches. First, highly saprophytic strains of *Fusarium* spp. should be screened from the naturally-occurring populations on wheat stubble in the field, and those with greater decomposition capabilities might be able to more readily degrade the lignified cellulose complex in sawfly-cut stems and subsequently kill overwintering larvae. Next, endophytic strains of *Fusarium* spp. with entomopathogenic properties should be

assessed with sawfly larvae feeding in growing wheat plants. Impacts on developing larvae could be dramatic due to the easy access for these *Fusarium* strains in the inner lumen of wheat stems. Third, factors that could influence the pathogenicity of *Fusarium* spp. to sawfly larvae, including tillage practices, wheat varieties, soil type and moisture, and fungicide applications, should be further studied to take advantage of this antagonistic relationship between *Fusarium* and sawfly. Next, other microorganisms involved in this system, such as *Paecilomyces* spp. that were isolated in the course of this research, could also be good candidates for controlling wheat stem sawflies. This should definitely be investigated under field and laboratory conditions. Finally, regional agricultural strategies, such as optimizing the spatial arrangements of dryland and irrigated wheat fields over large and contiguous areas, should be developed because of the potential to further suppress populations of wheat stem sawfly on a landscape scale.